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(57) Abstract

New biomaterials essentially constituted by esterified derivatives of hyaluronic acid or by cross-linked derivatives of hyaluronic acid for use in the surgical sector, particularly for use in the prevention of post-surgical adhesions.

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BIOMATERIALS FOR PREVENTING POST-SURGICAL ADHESIONS COMPRISED OF HYALURONIC ACID DERIVATIVES

Object of the Invention

The present invention concerns new biomaterials essentially constituted by esterified derivatives of hyaluronic acid or by cross-linked derivatives of hyaluronic acid for use in the surgical sector, particularly for use in the prevention of post-surgical adhesions.

Field of the Invention

Postoperative adhesion formation is a common complication in abdominal or pelvic surgery which may lead to a substantial morbidity. Many factors may influence the development of adhesions: mechanical trauma, chemical agents, drying of serosa in combination with blood, ischemia, infection and foreign material are all known to increase adhesion formation. Other causes are intraabdominal inflammatory diseases and congenital abnormalities. The pathophysiological mechanism still remain unclear, but a central common pathway in which peritoneal fibrinolysis plays an important role has been suggested.

The surgical trauma of the tissue causes the release of a serosanguinous exudate which forms a fibrinous bridge that persists several days during which cell growth occurs. If the exudate is not absorbed or lysed within this period, it becomes ingrown fibroblast and subsequent collagen deposition leads to the formation of a permanent scar connecting the two adjacent surfaces, called an adhesion. In conclusion, adhesion formation seems to be a result of an inflammatory response.

In this latter case, the research has mainly focused on the search for bioabsorbable materials with a short time of *in vivo* persistence, acting as a barrier to adhesion formation until healing has occurred; in order to obviate the problems caused by non-absorbable materials (infection, calcification of the implants, scar formation, etc.).

One particularly promising polymer is Hyaluronic Acid (HA), a component of extracellular matrix ubiquitously found within the human body. Hyaluronic Acid solutions have been shown to reduce postoperative adhesion formation after abdominal (Urman, B. et al., <u>Effect of Hyaluronic Acid on Postoperative Intraperitoneal Adhesions Formation in the Rat Model</u>, Fertil. Steril. 1991; 56:563; Shushan A. et al., Hyaluronic Acid for Preventing Experimental Postoperative-intraperitoneal Adhesions, J. Reprod. Med. 1994; 39:398) and orthopaedic operation (Hagberg, L, Gerdin, B., Sodium Hyaluronate as an adjunctive in adhesion prevention after flexor tendon surgery in rabbits, J. Hand. Surg. 1992; 17A:935).

Fidia Advanced Biopolymers has developed chemical derivatives of hyaluronic acid, i.e. internal esters (ACP series) and esters with non-active alcohols (HYAFF series) Rastrelli, A. et al., Hyaluronic Acid Esters, A New Class of Semisynthetic Biopolymers: Chemical and Physico-chemical Properties, Clinical Implant Materials,

Advanced in Biomaterials, G. Heinrike, V. Sollz and AJC Lee (Eds), Elsevier, Amsterdam 1990; 9:199-205, which display physico-chemical properties different from that of HA (i.e. higher residence time and ability to be manufactured to produce devices, but possess tolerability and biocompatibility properties typical of the original biological polymer). Moreover, these derivatives are characterized from a chemical and toxicological point of view.

The aim of the present invention has been to develop batches of ACP gel in an attempt to evaluate the effect in adhesion prevention.

The onset of adherences, or fibrous masses which form between adjacent tissues affected by trauma or ischemia following surgery, is still one of the most serious complications in numerous surgical procedures. A large number of methods have been proposed to avoid this complication, but the problem has remained mainly unsolved.

One proposed method has been the use of suspensions of dextran (diZerega G.S., "Contemporary adhesion prevention" Fertility and Sterility, Vol. 61, No. 2, February '94) injected into the peritoneal cavity after surgery. The clinical results of the use of such dextran solutions have been largely discordant. Moreover, the use of solutions of dextran has been accompanied by frequent complications, including edema, abdominal pain and dyspnea.

The use of barriers in the form of defined structures (e.g. meshes, membranes) (diZerega G.S., "Contemporary adhesion prevention" Fertility and Sterility, Vol. 61, No. 2, February '94) or viscous gels (Genzyme U.S. Patent No. 4,937,270 - U.S. Patent 5,017,229) placed between the injured organs has also been proposed. However, these barriers have generally proved ineffective because they provoke ischemic or inflammatory reactions due to the presence of foreign

bodies. The only materials currently approved for clinical use are barriers based on oxidized regenerated cellulose (Interceed®) and barriers based on expanded polytetrafluorine ethylene (e-PTFE) (Goretex® - U.S. Patent 4,478,665 and U.S. Patent 4,482,516) or polyethylene or polypropylene.

In addition to the fact that clinical investigations into the efficacy of such barriers have produced highly discordant results, it must also be noted that both of the aforesaid materials are associated with major contraindications. The use of barrier membranes of e-PTFE or polyethylene or polypropylene involves the implantation of a synthetic material which is foreign to the human body and not biodegradable, and which may require a second surgical operation to remove or reposition the barrier membrane because of undesirable inflammatory-type reactions.

In preclinical and clinical models, meshes based on oxidized regenerated cellulose have proved to be efficacious in preventing the formation of adherences, but only if their application is preceded by thorough hemostasis.

The use of viscous solutions of high-molecular-weight hyaluronic acid (HA) has, therefore, been proposed as an aid in the prevention of adherence (Grainger D.A. et al., "The use of hyaluronic acid polymers to reduce postoperative adhesions", J. of Gynecol. Surg., Vol. 7, No. 2, 1991; Hurman B. et al., "Effect of hyaluronic acid on postoperative intraperitoneal adhesion formation in the rat model", Fertility and Sterility, Vol. 56, No. 3, September 1991; Shushan A. et al., "Hyaluronic acid for preventing experimental postoperative intraperitoneal adhesions:, J. of Reproductive Med., Vol. 39, No. 5, May 1994; Mitchell J. D. et al., "Reduction in experimental pericardial adhesions using a hyaluronic acid bioabsorbable membrane", Eur. J. Cardio-thorac. Surg.,

8, 149-152, 1994). Hyaluronic acid as such, however, is characterized by very rapid absorption times which are incompatible with the residence time necessary to prevent adhesion. Moreover, natural hyaluronic acid cannot be processed and as such cannot be transformed into biomaterial form. In order to prolong its degradation times and enable it to be processed into various physical forms for use in different surgical sectors, esters of hyaluronic acid and cross-linked derivatives of hyaluronic acid have been developed. The preparation of esters of hyaluronic acid, wherein all or part of the carboxy groups are esterified, the preparation of cross-linked derivatives of hyaluronic acid, wherein part of the carboxy groups undergo cross-linking and their uses in the pharmaceutical, cosmetic and surgical sectors and in that of biodegradable plastic materials are described in U.S. Patents Nos. 4,851,521 and 4,956,353, EP 0 216 453 and EP 0 341 745.

Summary of the Invention

The present invention provides biomaterials for use in the prevention of post-surgical adhesions. The biomaterials are comprised of benzyl esters of hyaluronic acid and/or internally cross-linked derivatives of hyaluronic acid and may be in the form of gels, membranes, woven tissues or meshes and nonwoven tissues.

Brief Description of the Drawings

Figures 1-10 are graphs of the results of adhesion studies in rat animal models.

Detailed Description of the Invention

The present invention, therefore, describes the preparation of healthcare and surgical articles based on a benzyl ester of hyaluronic acid or on cross-linked

derivatives of hyaluronic acid, used singly or in mixtures with one another, characterized by high biocompatibility and transformable into physical forms which make them suitable for various uses in surgery, including laproscopical surgery. The materials are also completely biodegradable and do not need to be removed from the application site, thus avoiding a second surgical operation. When prepared in the form of gels, the cross-linked derivatives present materials with significantly greater viscosity than the unmodified polymer and with variable degradation times. Moreover, both the benzyl ester-based materials and the cross-linked derivative-based materials of the present invention can be in the form of membranes, woven tissues or meshes and nonwoven tissues (prepared according to procedures *per se* described in U.S. 4,851,521; U.S. 4,956,353,; WO 93/11804; WO 93/11803; WO 94/17837 and EP 0 341 745) and are characterized by the following technical specifications:

- the membranes vary in thickness between 10μm and 1.5 mm, especially 20-50 μm;
- the tissues or meshes vary in thickness between 200μm and 1.5 mm;
- the nonwoven tissues are essentially characterized by a basis weight which varies between 20 g/m^2 and 500 g/m^2 and by a thickness of between 0.2 mm and 5 mm, especially <1 mm.

These materials can be used singly or in association with one another or with other materials constituted by synthetic polymers (e.g. gels based on cross-linked hyaluronic acid + polypropylene, or membranes essentially constituted by esterified derivatives of HA + polypropylene or membranes comprised of esterified derivatives of HA, coated with a gel of auto-crosslinked HA).

Indeed, the present invention also concerns the use of composite materials in the form of gels (for the

cross-linked derivatives), membranes, woven or nonwoven tissues, essentially constituted by the benzyl esters or cross-linked derivatives of hyaluronic acid in association with nonbiodegradable materials in the form of meshes or membranes or nonwoven tissues such as e-PTFE, polyethylene, polypropylene, polyester (Dacron®). The present invention, therefore concerns a new class of healthcare and surgical articles to be used in the field of surgery for the prevention of the formation of post-surgical adherence.

Materials

As noted above, the present invention is characterized by materials comprised of derivatives of hyaluronic acid, especially benzyl ester derivatives and internally cross-linked derivatives.

The term "hyaluronic acid" (also referred to as "HA" hereinafter) is used in literature to designate an acidic polysaccharide with various molecular weights constituted by resides of D-glucuronic acid and N-acetyl-D-glucosamine, which naturally occur in cellular surfaces, in the basic extracellular substances of the connective tissues of vertebrates, in the synovial fluid of joints, in the vitreous humor of the eye, in the tissue of the human umbilical cord and in cocks' combs.

Hyaluronic acid plays an important role in the biological organism, firstly as a mechanical support of the cells of many tissues, such as the skin, the tendons, the muscles and cartilage and it is therefore the main component of the extracellular matrix. But hyaluronic acid also performs other functions in the biological processes, such as the hydration of tissues, lubrication, cellular migration, cell function and differentiation. (See for example, A. Balazs et al., Cosmetics & Toiletries, No. 5/84, pages 8-17). Hyaluronic acid may be extracted from the above-mentioned natural tissues, such as cocks' combs, or also

from certain bacteria. Today, hyaluronic acid may also be prepared by microbiological methods. The molecular weight of whole hyaluronic acid obtained by extraction is in the region of 8-13 million. However, the molecular chain of the polysaccharide can be degraded quite easily under the influence of various physical and chemical factors, such as mechanical influences or under the influence of radiation, hydrolyzing, oxidizing or enzymatic agents. For this reason, often in the ordinary purification procedures of original extracts, degraded fractions with a lower molecular weight are obtained. (See Balazs et al., cited above). Hyaluronic acid, its molecular fractions and the respective salts have been used as medicaments and their use is also proposed in cosmetics (see for example, the above-mentioned article by Balazs et al., and the French Patent No. 2478468).

Although the term "hyaluronic acid" is commonly used in an improper sense, meaning, as can be seen from above, a whole series of polysaccharides with alternations of residues of D-glucuronic acid and N-acetyl-D-glucosamine with varying molecular weights or even degraded fractions of the same, and although the plural form "hyaluronic acids" may seem more appropriate, the discussion herein shall continue to use the singular form to refer to hyaluronic acid in its various forms including its molecular fractions, and the abbreviation "HA" will also often be used to describe this collective term.

1. <u>The Benzyl Ester Derivatives</u>:

The first preferred material of the invention is based on the benzyl ester of hyaluronic acid, particularly the 80-100% esters wherein 80% to 100% of the HA carboxyl groups are esterified. Those benzyl esters wherein 80-99% of the HA carboxyl groups are esterified with a benzyl group are referred to as

"partial esters", because only a portion of the carboxyl groups are esterified and the remaining carboxyl groups are either free or salified with an alkaline or alkaline earth metal, such as sodium, calcium or potassium.

Most preferred for the biomaterials of the invention are so-called "total" benzyl esters wherein all of the HA carboxy groups are esterified. In these total esters, all of the HA carboxy groups may be esterified with a benzyl group (also referred to as HYAFF 11) or a portion (75 to 99%) may be esterified with a benzyl group and all of the remaining carboxyl groups are esterified with the lipid chain/alkyl residue from a C_{10-20} aliphatic alcohol to produce what may be referred to as "mixed" esters. Of these aliphatic alcohols, palmitic alcohol (C_{16}-hexadecyl) and stearic alcohol (C_{10} octadecyl) are most preferred. These mixed esters may also be in the form of partial esters, that is, derivatives wherein a portion (75 to 99%) of the carboxyl groups are esterified with a benzyl group and some, but not all, of the remaining carboxyl groups are esterified with the C_{10}-C_{20} aliphatic alcohol. Of these, most preferred are those which are at least 75% benzyl esterified and at least 5% esterified with a C_{10}-C_{20} aliphatic alcohol.

The benzyl esters of hyaluronic acid according to the invention may be prepared by methods known *per se* for the esterification of carboxylic acids, for example by treatment of free hyaluronic acid with the alcohol (benzyl and/or C_{10}-C_{20} alcohol) in the presence of catalyzing substances, such as strong inorganic acids or ionic exchangers of the acid type, or with an etherifying agent capable of introducing the desired alcoholic residue in the presence of inorganic or organic bases.

The benzyl hyaluronic esters may, however, be prepared to advantage according to a particular method described in EP 0 216 453. This method consists of

treating a quaternary ammonium salt of hyaluronic acid with an etherifying agent, preferably in an aprotic organic solvent.

For the preparation of the benzyl esters it is possible to use hyaluronic acids of any origin, such as for example, the acids extracted from the above mentioned natural starting materials, for example, from cocks' combs. The preparation of such acids is described in literature: preferably, purified hyaluronic acids are used. According to the invention, especially used are hyaluronic acids comprising molecular fractions of the integral acids obtained directly by extraction of the organic materials with molecular weights varying within a wide range, for example, from about 90%-80% (M = 11.7 - 10.4 million) to 0.2% (M = 30,000) of the molecular weight of the integral acid having a molecular weight of 13 million, preferably between 5% and 0.2%. Such fractions may be obtained with various procedures described in literature, such as by hydrolyzing, oxidizing, enzymatic or physical procedures, such as mechanical or radiational procedures. Primordial extracts are therefore often formed during these same purification procedures (for example, see the article by Balazs et al., quoted above in "Cosmetics & Toiletries"). The separation and purification of the molecular fractions obtained are brought about by known techniques, for example by molecular filtration.

One fraction of purified HY suitable for use according to the invention is for example that known as "non-inflammatory-NIF-NaHA sodium hyaluronate described by Balazs in the booklet "Healon" - A guide to its use in Ophthalmic Surgery, D. Miller & R. Stegmann, eds. John Wiley & Sons, N.Y., 81983: p 5.

Particularly important as starting materials for the benzyl ester are two purified fractions obtainable from hyaluronic acid, for example the ones extracted from cocks' combs, known as "Hyalastine" and

"Hyalectin". The fraction Hyalastine has an average molecular weight of about 50,000 to 100,000 while the fraction Hyalectin has an average molecular weight of between about 500,000 and 730,000. A combined fraction of these two fractions has also been isolated and characterized as having an average molecular weight of about 250,000 to about 350,000. This combined fraction may be obtained with a yield of 80% of total hyaluronic acid available in the particular starting material, while the fraction Hyalectin may be obtained with a yield of 30% and the fraction Hyalastine with a yield of 50% of the starting HY. The preparation of these fractions is described in EP 0 138 572.

The following Examples describe the preparation of the benzyl esters of HA.

Example 1 - Preparation of the Benzylester of Hyaluronic Acid (HY).

12.4 g of HY tetrabutylammonium salt with a molecular weight of 170,000 corresponding to 20 m.Eq. of a monomeric unit are solubilized in 620 ml of dimethylsulfoxide at 25°, 4.5 g (25 m.Eq.) of benzyl bromide and 0.2 g of tetrabutylammonium iodide are added, the solution is kept for 12 hours at 30°.

The resulting mixture is slowly poured into 3,500 ml of ethyl acetate under constant agitation. A precipitate is formed which is filtered and washed four times with 500 ml of ethyl acetate and finally vacuum dried for twenty four hours at 30°.

9 g of the benzyl ester product in the title are obtained. Quantitative determination of the ester groups is carried out according to the method described on pages 169-172 of Siggia S. and Hann J.G. "Quantitative organic analysis via functional groups" 4th edition, John Wiley and Sons.

Example 2 - Preparation of the benzyl ester of hyaluronic acid (HY)

3 g of the potassium salt of HY with a molecular weight of 162,000 are suspended in 200 ml of dimethylsulfoxide; 120 mg of tetrabutylammonium iodide and 2.4 g of benzyl bromide are added.

The suspension is kept in agitation for 48 hours at 30°C. The resulting mixture is slowly poured into 1,000 ml of ethyl acetate under constant agitation. A precipitate is formed which is filtered and washed four times with 150 ml of ethyl acetate and finally vacuum dried for twenty four hours at 30°.

3.1 g of the benzyl ester product in the title are obtained. Quantitative determination of the ester groups is carried out according to the method described on pages 1698-172 of Siggia S. and Hanna J.G. "Quantitative organic analysis via functional groups" 4th Edition, John Wiley and Sons.

Example 3 - Preparation of a Hyaluronic Acid Derivative With 75% of its Carboxyl Functions Esterified With Benzyl Alcohol and the Remaining 25% Esterified With Octadecyl Alcohol (Stearyl Alcohol, $CH_3-(CH_2)_{16}-CH_2-OH$)

6.21 g of tetrabutyl ammonium salt of hyaluronic acid with a molecular weight of 180,000 Daltons (10 mEq) are solubilized in 248 ml of dimethylsulfoxide (DMSO) at room temperature.

This solution is supplemented with 0.89 ml of benzyl bromide (7.5 mEq) and the solution is left to stand at 30°C for 12 hours. The solution is then cooled to room temperature and supplemented with 0.83 g of octadecyl bromide (2.5 mEq). The solution is heated to 30°C for 24 hours. A 2.5% solution (w/w) of NaCl in water is then added and the resulting mixture is poured into 750 ml of acetone, stirring the while. A precipitate is formed which is filtered and washed three times in 100 ml of acetone/water 5:1, three times with 100 ml of acetone and then dried in a high vacuum for 24

hours at 30°C. 5.1 grams of the desired product are thus obtained. Quantitative determination of the benzyl alcohol and hexadecyl alcohol content is performed by gas chromatography following alkaline hydrolysis. The total content of ester groups is quantified according to the saponification method described on pages 169-172 of "Quantitative organic analysis via functional group" Fourth Edition (John Wiley and Sons Publication).

Example 4 - Preparation of a Hyaluronic Acid Derivative With 75% of its Carboxyl Functions Esterified With Benzyl Alcohol and the Remaining 25% Esterified With Hexadecyl Alcohol (Cetyl Palmityl Alcohol, $CH_3-(CH_2)_{14}-CH_2-OH$)

6.21 g of tetrabutyl ammonium salt of hyaluronic acid with a molecular weight of 180,000 Daltons (10 mEq) are solubilized in 248 ml of dimethylsulfoxide (DMSO) at room temperature.

This solution is supplemented with 0.89 ml of benzyl bromide (7.5 mEq) and the solution is left to stand at 30°C for 12 hours. The solution is then cooled to room temperature and supplemented with 0.76 g of hexadecyl bromide (2.5 mEq). The solution is heated to 30°C for 24 hours. A 2.5% solution (w/w) of NaCl in water is then added and the resulting mixture is poured into 750 ml of acetone, stirring the while. A precipitate is formed which is filtered and washed three times in 100 ml of acetone/water 5:1, three times with 100 ml of acetone and then dried in a high vacuum for 24 hours at 30°C. Five grams of the desired product are thus obtained. Quantitative determination of the benzyl alcohol and hexadecyl alcohol content is performed by gas chromatography following alkaline hydrolysis. The total content of ester groups is quantified according to the saponification method described on pages 169-172 of "Quantitative organic analysis via functional group" Fourth Edition (John Wiley and Sons Publication).

2. **The Internal Cross-Linked Hyaluronic Acid Derivatives:**

The cross-linked hyaluronic acid derivatives used in the materials of the present invention are described in EP 0 341 745. These cross-linked derivatives are inter and/or intramolecular esters of hyaluronic acid wherein a part of the carboxy groups are esterified with hydroxyl groups of the same molecule and/or of different molecules of hyaluronic acid, thus forming lactone or intermolecular ester bonds. These "inner" esters in which there is no intervention by OH groups of other alcohols, can also be defined as "auto-crosslinked hyaluronic acid" since the formation of a mono- or polymolecular cross-link is the consequence of the above-mentioned internal esterification. The adjective "cross-linked" refers to the crosswise connections between the carboxyls and hydroxyls of the hyaluronic acid molecules.

The auto-crosslinked products are particularly partial inner esters wherein the percentage of "cross-links" varies preferably between 0.5 to 20%, especially 4.5/5.0 % of the number of carboxy groups in the hyaluronic acid. In the preparation process, the carboxy groups of the HA molecule are activated by the addition of substances capable of inducing such activation. The unstable intermediate products obtained from the activation reaction separate spontaneously, either after the addition of catalysts and/or following a rise in temperature, forming the above mentioned inner ester bonds with hydroxyls of the same or other hyaluronic acid molecule. According to the degree of inner esterification desired, either all or an aliquot part of the carboxy functions are activated (the aliquot part being obtained by using an excess of activating substances or by suitable dosing methods).

The carboxy groups to be converted into inner ester groups can be activated starting from hyaluronic acid

containing free carboxy groups, or, preferably, from HA containing salified carboxy groups, for example, metal salts, preferably alkaline or alkaline earth metals, and above all with quaternary ammonium salts, such as those described hereafter. Salts with organic basis such as amines can, however, also be used as starting substances.

Methods for the activation of free or salified carboxy groups are *per se* known, particularly in the field of peptide synthesis, and those skilled in the art can easily determine which method is the most suitable, especially whether or not to use the starting substances in their free or salified form. Activation methods *per se* known for peptide synthesis procedures and useful in the preparation procedures of the present invention are described, for example, in Bodanszky, M., In search of new methods in peptide synthesis, Int. J. Peptide Protein Res. 25, 1985, 449-474; and Gross, E. et al, The Peptides, Analysis Synthesis, Biology, Academic Press, Inc., 1979, Vol. 1, Chapter 2. According to such procedures, a carboxyl component is activated, that is, a carboxyl component is converted to a reactive form. Such activation typically involves a reaction between an acid and an activating agent according to the scheme:

$$R\text{-}COOH \rightarrow R\text{-}\overset{\displaystyle O}{\overset{\|}{C}}\text{-}X,$$

wherein X is an electron withdrawing moiety. Most activated derivatives of carboxylic acids, therefore, are mixed anhydrides, including in the broad sense also acid azides and acid chlorides which can be considered mixed anhydrides of hydrazoic acid and HCℓ as the activating agents. In addition, activation of a carboxyl component can be accomplished by the formation of intermediate "activated esters". These "activated esters" can be of various types, but particularly useful "activated esters" are those prepared by use of dicyclohexylcarbodiimide, p-nitrophenyl esters,

trichlorophenyl esters, pentachlorophenyl esters, and O-acyl derivatives of hydroxylamines, particularly esters of N-hydroxysuccinimide.

All of these various types of activation procedures are useful in the preparation of the cross-linked HA of the invention, as all of these procedures can be characterized as importantly involving the reaction of a carboxyl group with an activating agent which essentially results in the formation of a substituent group that is easily reactive with a hydroxyl group so as to easily form the inner ester bonding characteristic of the products of the invention, the number of carboxy functions to be converted into inner esters in proportion to the number of activated carboxy functions and this number depends on the quality of the activating agent used.

The preferred procedure for preparation of cross-linked HA is therefore characterized by treating HA, having free or salified carboxy groups, with an agent which activates the carboxy function, possibly in the presence of an auxiliary agent favoring the formation of intermediate activated derivatives and/or a tertiary organic or inorganic base, exposing the mixture to heating or irradiation (particularly with UV light), and if desired, by salifying free carboxy groups or by freeing salified carboxy groups. Of the substances able to activate the carboxy group, the conventional ones described in literature can be used, for example, those usually used in the synthesis of peptides, except however those which would have the effect of altering or destroying the molecular structure of the starting HA, such as those used for the formation of carboxyl halides. Preferred substances which lead to the formation of activated esters are those, such as, carbodiimides, dicyclohexylcarbodiimide, benzyl-isopropylcarbodiimide, benzyl-ethyl-carbodiimide; ethoxyacetylene; Woodward's reagent (N-ethyl-5-

phenylisoxazolium-3-sulfonate) or halogen derivatives from aliphatic, cycloaliphatic or aromatic hydrocarbons, or from heterocyclic compound with halogen made mobile by the presence of one or more activating groups, such as chloroacetonitryl and especially the salts of 2-chloro-N-alkylpyridine, such as chloride of 2-chloro-N-methyl-pyridine or other alkyl derivatives with inferior alkyl groups, such as those with up to 6 carbon atoms. In the place of chloride derivatives, other halogen derivatives can of course be used, such as bromide derivatives.

This activation reaction can be carried out in organic solvents, especially aprotic solvents such as dialkylsulfoxides, dialkylcarboxylamides, such as in particular lower alkyl dialkylsulfoxides, particularly dimethylsulfoxide, polymethylene sulfoxides, such as tetramethylene sulfoxide, dialkyls or polymethylene sulfones, such as tetramethylene sulfone, sulfolane and lower alkyl dialkyamides of lower aliphatic acids in which the alkyl groups have a maximum of six carbon atoms, such as dimethyl or diethyl formamide or dimethyl or diethyl acetamide. Other solvents may also be used, however, and these need not always be aprotic, such as alcohols, ethers, ketones, esters, such as lower aliphatic dialkyloxyhydrocarbides, such as dimethoxyethane and especially aliphatic or heterocyclic alcohols and ketones with a low boiling point, such as lower N-alkyl-pyrrolidones, such as N-methylpyrrolidone or N-ethyl-pyrrolidone, hexafluorisopropanol and trifluoroethanol. If halogen derivatives are used as carboxyl-activating substances, especially in the form of salts, such as the above-mentioned 2-chloro-N-methylpyridinium chloride, it is better to use a metal salt or a salt of the organic base of the starting polysaccharide, especially one of the quaternary ammonium salts described hereafter, such as tetrabutyl ammonium salt. These salts have the special advantage

of being very soluble in the abovesaid organic solvents in which the cross-linking reaction is best effected, thus guaranteeing an excellent yield It is advisable to add to the mixture a substance capable of subtracting acid, such as organic bases, carbonates, bicarbonates or alkaline or alkaline earth acetates, or organic bases and especially tertiary bases such as pyridine and its homologues, such as collidine, or aliphatic amine bases, such as triethylamine or N-methyl-piperazine.

The use of quaternary ammonium salts represents a particularly advantageous procedure. Such ammonium salts are well known and are prepared in the same way as other known salts. They derive from alkyls having preferably between 1 and 6 carbon atoms. It is preferable to use tetrabutyl ammonium salts. One variation in the procedure in which quaternary ammonium salts are used, consists in reacting an alkaline salt, for example, sodium or potassium salt, in the presence of catalyzing quantity of a quaternary ammonium salt, such as tetrabutylammonium iodide.

The substances which catalyze activation of the carboxy groups to be added to the activating agents are reported in literature and these too are preferably bases such as those mentioned previously. Thus, for example, when the carboxy groups are activated with isothiazoline salts it is preferable to add some triethylamine to the reaction mixture.

The reaction of formation of activated intermediates, such as and especially esters, is carried out at the temperature recommended in literature and this temperature can, however, be varied should circumstances require as can be easily determined by one skilled in the art. The formation of inner ester bonds can come about within a fairly wide temperature range, for example between 0° and 150°, preferably room temperature or slightly above, for example between 20° and 75°. Raising the temperature favors the formation

of inner ester bonds, as does exposure to radiations of suitable wavelength, such as ultraviolet rays.

The substrate of hyaluronic acid can be of any origin, and can be of the various types discussed above. The preferred HA starting materials are those with an average molecular weight of 150,000 to 730,000, especially 150,000 to 450,000 daltons.

In addition, the amount of internal cross-linking can vary, but preferred materials according to the invention utilize HA cross-linked to a degree of 4.5 to 5.0% of the carboxyl groups.

The following Examples describe the preparation of useful cross-linked HY products for making the materials of the invention.

Example 5 - Preparation of 1% Cross-Linked Hyaluronic Acid (HY)

Product description:

1% of carboxy groups used in internal esterification.

99% of carboxy groups salified with sodium.

6.21 g of HY tetrabutylammonium salt with a molecular weight of 170,000 corresponding to 10 mEq of a monomeric unit are solubilized in 248 ml of DMSO at 25°C, 0.01 g (0.1 mEq) of triethylamine are added.

Example 6 - Preparation of 5% Cross-Linked Hyaluronic Acid

Product description:

5% of carboxy groups used in internal esterification.

95% of carboxy groups salified with sodium.

6.21 g of HY tetrabutylammonium salt with a molecular weight of 85,000 corresponding to 10 mEq of a monomeric unit are solubilized in 248 ml of DMSO at 25°C, 0.051 gr (0.5 mEq) of triethylamine are added and the resulting solution is agitated for 30 minutes.

A solution of 0.128 gr (0.5 mEq) of 2-chloro-1-methyl pyridinium iodide in 60 ml of DMSO is slowly added drop by drop over a time interval of 1 hour and the mixture is kept for 15 hours at 30°C.

A solution formed by 100 ml of water and 2.5 gr of sodium chloride is then added and the resulting mixture is then poured slowly into 750 ml of acetone, maintaining continual agitation. A precipitate is formed which is then filtered and washed three times in 100 ml of acetone water 5:1 and three times with 100 ml of acetone and lastly vacuum-dried for 24 hours at 30°.

3.95 grs of the title compound are obtained. Quantitative determination of the ester groups is carried out according to the saponification method described on pp. 169-172 of "Quantitative Organic Analysis Via Functional Groups", 4th Edition, John Wiley and Sons Publication.

<u>Example 7: Preparation of 10% Cross-linked Hyaluronic Acid (HY)</u>

Product description:

10% of carboxy groups used in internal esterification.

90% of carboxy groups salified with sodium.

6.21 g of HY tetrabutylammonium salt with a molecular weight of 620,000 corresponding to 10 mEq of a monomeric unit are solubilized in 248 ml of DMSO at 25°C. 0.101 gr (1.0 mEq) of triethylamine is added and the resulting solution is agitated for 30 minutes.

A solution of 0.255 gr (1.0 mEq) of 2-chloro-1-methyl-pyridinium iodide in 60 ml of DMSO is slowly added drop by drop over a time interval of 1 hour and the mixture is kept for 15 hours at 30°C.

A solution formed by 100 ml of water and 2.5 gr of sodium chloride is then added and the resulting mixture is then poured slowly into 750 ml of acetone, maintaining continual agitation. A precipitate is

formed when is then filtered and washed three times in 100 ml of acetone water 5:1 and three times with 100 ml of acetone and lastly vacuum-dried for 24 hours at 30°.

3.93 grs of the title compound are obtained. Quantitative determine of the ester groups is carried out according to the saponification method described on pp. 169-172 of "Quantitative Organic Analysis Via Functional Groups", 4th Edition, John Wiley and Sons Publication.

Example 8: Preparation of 10% Cross-linked Hyaluronic Acid (HY)

Product Description:

10% of carboxy groups used in internal esterification.

90% of carboxy groups salified with sodium.

6.21 gr of HY tetrabutylammonium salt with a molecular weight of 170,000 corresponding to 10 mEq of a monomeric unit are solubilized in 248 ml of DMSO at 25°C, 0.118 gr (1 mEq) of pyridine chloride are added and the resulting solution is agitated for 30 minutes.

A solution of 0.16 g (mEq) of N-benzyl-N-ethyl carbodiimide in 20 ml of DMSO is slowly added drop by drop over a time interval of 1 hour and the mixture is kept at a temperature of 30° for 45 hours.

A solution made up of 100 ml of water and 2.5 of sodium chloride is added and the resulting mixture is slowly poured into 750 ml of acetone, maintaining continual agitation. A precipitate is formed which is then filtered and washed three times with 100 ml of acetone/H_2O 5:1 and three times with 100 ml of acetone finally vacuum-dried for 24 hours at a temperature of 30°.

3.9 grs of the title compound are obtained. Quantitative determination of the total ester groups is carried out according to the saponification method described on pp. 169-172 of "Quantitative Organic

Analysis Via Functional Groups", 4th Edition, John Wiley and Sons Publication.

3. **Preparation of the Biomaterials:**

The following Examples describe the preparation of surgical/healthcare products according to the invention which comprise the total benzyl ester of HA or an auto-crosslinked HA derivative, or combinations thereof. As noted above, procedures for the preparation of the membranes, woven tissues, woven meshes, and nonwoven tissues are described in U.S. 4,851,521; U.S. 4,956,353, WO 93/11804; WO 93/11803; WO 94/17837 and EP 0 341 745.

Example 9 - Preparation of a Product Based on HYAFF 11 + Polypropylene Mesh

A solution of HYAFF 11 in DMSO is prepared (110 mg/ml). Once solubilization is complete, the solution is filtered through a 20 μm filter cloth and degassed by leaving it to stand in a vacuum for 2 hours. 5 ml of the solution are poured out and spread onto a glass plate, after which the polypropylene mesh (preferably 6x11 cm) is placed on top and a further 10 ml of the solution is poured over it. This is spread evenly over the mesh and any excess is eliminated.

The glass plate is immersed in a bath containing ethanol/H_2O (90:10) for 5 hours to allow the preparation to coagulate and the plate to become detached; the preparation is then immersed in a bath of absolute ethanol for 16 hours. It is then dried on a plate in a vacuum for 30 minutes at 63°C.

Example 10: Preparation of a Product Based on Woven Tissue of HYAFF 11 Covered With a Film of HYAFF 11

A solution of HYAFF 11 in DMSO is prepared (110 μg/ml). Once solubilization is complete, the solution is filtered through a 20 μm filter cloth and degassed by leaving it to stand in a vacuum for 2 hours. 5 ml of

the solution are poured out and spread onto a glass plate, after which the HYAFF 11 gauze (10x20 cm) is placed on top, taking care to make it adhere without any creases or air bubbles, and a further 10 ml of the solution is poured over it. This is spread evenly over the gauze and any excess is eliminated.

The glass plate is immersed in a bath containing ethanol for 30 minutes to allow the preparation to coagulate and the plate to become detached. The preparation is then left in ethanol for 16 hours and dried on a plate in a vacuum at 63°C for 30 minutes.

Example 11 - Membrane of HYAFF 11 with Reinforcement of HYAFF 7

A composite membrane comprising the hyaluronic acid benzyl ester HYAFF 11, i.e., hyaluronic acid esterified 100% with benzyl alcohol, with a mesh reinforcement comprising the hyaluronic acid ethyl ester HYAFF 7, i.e., hyaluronic acid esterified 100% with ethanol, basis weight 14 mg/cm², 0.25 mm thick, minimum tensile strength at break and elongation when dry, 400 Kg/cm² and 7%, respectively, minimum tensile strength and elongation when wet, 50 Kg/cm² and 55%, respectively, tear strength when dry, 90 Kg/cm² tear strength when wet, 50 Kg/cm² was produced according to the following procedure.

The HYAFF 7 mesh was obtained starting with a solution of HYAFF 7 dimethylsulfoxide at a concentration of 125 mg/ml. The solution is fed by a gear metering pump into a spinneret for wet extrusion composed of 100 holes each measuring 65 microns in diameter.

The extruded multiple thread is passed into a coagulation bath containing absolute ethanol and is then moved over transporting rollers into three successive rinsing baths, also containing absolute ethanol. The ratio between the speed of the third roller (III) and the speed of the first roller (I) is called the drafting

ratio, and has a value of 1.05, while the speeds of the single rollers are: 23 rpm (roller I), 24 rpm (rollers II and III), 25 rpm (roller IV). Once the multiple thread has been passed through the rinsing baths it is dried with warm air at a temperature of 45°C and wound onto a winding frame (8). The thread is 237 denier. The multiple thread is then twisted 135 times per meter and woven on a loom into a smooth knitted fabric with a gauge of 14. From the loom the fabric is fed through a calendar, which thins it down. Figure 2 shows the mesh which results from said process.

The polymeric matrix is applied by two airbrushes which spray a solution of HYAFF 11 in dimethylsulfoxide at a concentration of 40 mg/ml. The mesh thus sprayed is passed into a coagulation bath containing absolute ethanol, into a rinsing chamber containing pure, distilled water and into a special drying chamber with a temperature of 50°C (17).

Example 12 - Nonwoven Fabric Comprised of HYAFF 11

A nonwoven fabric comprising hyaluronic acid benzyl ester HYAFF 11, weighing 40 gr/mq, 0.5 mm thick, was produced by the following procedure.

A solution of HYAFF 11 in dimethylsulfoxide at a concentration of 135 mg/ml is prepared in a tank and fed by a gear metering pump into a spinneret for wet extrusion composed of 3000 holes each measuring 65 microns.

The extruded mass of threads passes into a coagulation bath containing absolute ethanol. It is then moved over transporting rollers into two successive rinsing baths containing absolute ethanol. The drafting ratio of the first roller is set at zero while the drafting ratio between the other rollers is set at 1.05. Once it has been passed through the rinsing baths, the hank of threads is blown dry with hot air at 45°-50°C and cut with a roller cutter into 40 mm fibers.

The mass of fibers thus obtained is tipped into a chute leading to a carding/cross lapping machine from which it emerges as a web, 1 mm thick and weighing 40 mg/mq. The web is then sprayed with a solution of HYAFF 11 in dimethylsulfoxide at 80 mg/ml, placed in an ethanol coagulation bath, in a rinsing chamber, and lastly in a drying chamber.

The final thickness of the material is 0.5 mm.

Example 13 - Nonwoven Fabric Comprised of HYAFF 11 and HYAFF 7

A nonwoven fabric weighing 200 gr/mq and 1.5 mm thick comprising a mixture of the ethyl ester of hyaluronic acid, HYAFF 7, and of hyaluronic acid benzyl ester, HYAFF 11, in equal quantities, was obtained by the following procedure.

Fibers of HYAFF 7 and HYAFF 11, measuring 3 mm in length, obtained by the spinning process described in Example 10 were thoroughly mixed in a spiral mixer. The mixture of fibers was fed into a carding machine from which it emerged as a 1.8 mm thick web weighing 200 gr/mq.

The web was put through a needle punching machine, which transformed it into a 1.5 mm thick unwoven fabric weighing 200 gr/mq, with the two materials perfectly mixed together.

Example 14 - Nonwoven Fabric of Partial and Total Benzyl Ester

A nonwoven fabric weighing 40 gr/mq and 0.5 mm thick comprising a mixture of hyaluronic acid benzyl ester, HYAFF 11, and a partial (75%) benzyl ester of hyaluronic acid, HYAFF 11p75, in equal percentages, was produced by the following procedure.

HYAFF 11p75 is prepared as follows. 10 g of hyaluronic acid tetrabutylammonium salt, mw = 620.76, equal to 16.1 nmole, are solubilized in a mixture of N-

methyl pyrrolidone/H_2O, 90/10, 2.5% in weight, to obtain 400 mls of solution. The solution is cooled to 10°C, then purified N_2 is bubbled through it for 30 minutes. This is then esterified with 1.49 ml (equal to 12.54 mmole) of benzyl bromide. The solution is gently shaken for 60 hours at 15-20°C.

Subsequent purification is achieved by precipitation in ethyl acetate following the addition of a saturated solution of sodium chloride, and subsequent washings with a mixture of ethyl acetate/absolute ethanol, 80/20. The solid phase is separated by filtration, and treated with anhydrous acetone. 6.8 g of product are thus obtained, equal to a yield of about 95%.

Fibers of HYAFF 11 and HYAFF 11p75, 40 mm long, obtained by the process described in Example 1, were thoroughly mixed in a spiral mixer.

The mixed fibers were fed into a carding machine from which they emerged as a 1 mm thick web weighing 40 mg/mq. The web was then sprayed with a solution of HYAFF 11 in dimethylsulfoxide at 80 mg/ml, placed in an ethanol coagulation bath, then in a rinsing chamber containing water or a mixture of water and ethanol in a ratio of from 10 to 95% ethanol, and finally in a drying chamber.

The material has a final thickness of 0.5 mm, and the fibers of HYAFF 11 and HYAFF 11p75 are perfectly mixed and adhered together.

<u>Example 15 - Multilayer Nonwoven Tissue Based on HYAFF 11</u>

A multilayer nonwoven tissue composed of a layer of hyaluronic acid benzyl ester, HYAFF 11, and a layer of nonwoven viscose (Jettex 2005 from ORSA), basis weight 80 g/mq, thickness 2 mm, and water absorption percentage 560% by weight, was obtained by the following procedure.

The layer which comes into contact with the skin comprises fibers of HYAFF 11 produced by the wet-spinning technique in the form of 30 g/mq sheet. Fibers were made into sheets.

This layer is joined by stitching to a second layer of nonwoven viscose tissue with a basis weight of 30 g/mq.

The final nonwoven product thus comprises two perfectly adhered layers with a total basis weight of 80 g/mq, a thickness of 2 mm, and a water absorption percentage of 560% by weight.

Example 16 - Multilayer Nonwoven Tissue Based on HYAFF 11

A multilayer nonwoven tissue comprising a mixed layer of hyaluronic acid benzyl ester, HYAFF 11, and calcium alginate in a 1:1 ratio and a reinforcing nonwoven tissue of polypropylene (spunbonded nonwoven base, 50 g/mq from NEUBERGER) with a basis weight of 70 g/mq, a thickness of 1.5 mm, and a water absorption percentage of 450% by weight, was obtained by the following procedure.

Fibers of HYAFF 11 and calcium alginate, 40 mm in length, obtained by conventional wet-spinning techniques, were mixed, made into a 20 g/mq sheet, and joined by stitching to a spunbonded nonwoven tissue with a basis weight of 50 g/mq.

The resulting material comprises two layers of nonwoven tissue with a total basis weight of 70 g/mq, a thickness of 1.5 mm, and a water absorption percentage of 450% by weight.

Example 17 - Multilayer Nonwoven Tissue Based on HYAFF 11

A multilayer nonwoven tissue comprising a layer of hyaluronic acid benzyl ester HYAFF 11 and a layer of polyurethane foam such as LYOBEND (from DELCON) with a basis weight of 100 g/mq, a thickness of 6 mm, and a water absorption percentage of 860% by weight, was obtained by the following procedure.

The layer which comes into contact with the skin comprises fibers of HYAFF 11 produced by the wet-spinning technique and made into a 45 g/mq sheet which is joined by stitching to a second layer of polyurethane foam.

The resulting nonwoven product comprises two perfectly adhered layers with a total basis weight of 100 g/mq, a thickness of 6 mm, and a water absorption percentage of 860% by weight.

Example 18 - Preparation of a Membrane Made of a Derivative of Hyaluronic Acid with 80% of the Carboxy Functions Esterified with Benzyl Alcohol (C_6H_5-CH_2-OH), 10% of the Carboxy Functions Involved in the Formation of Inner Ester Bonds and the Remaining 10% Salified with Sodium

6.21 gr of the tetrabutylammonium salt of hyaluronic acid with a molecular weight of 180,000 Daltons (10 mEq) are solubilized in 248 ml of dimethylsulfoxide (DMSO) at ambient temperature. To this solution are added 0,951 ml of benzyl bromide (8.0 mEq) and the solution is left to stand for 12 hours at 30°C. 0.101 gr of triethylamine (1.0 mEq) are added and the solution is stirred for 30 minutes. A solution of 0.255 gr (1.0 mEq) of 2-chloro-1-methyl-pyridine iodide in 60 ml of DMSO is added and the mixture is left to stand for 15 hours at 30°C.

A 2.5% solution (w.v) of NaCl in water is added and the resulting mixture is poured into 750 ml of acetone, while stirred. A precipitate is formed which is filtered and washed three times in 100 ml of

acetone/water 5:1, three times with 100 ml of acetone and finally vacuum-dried for 24 hours at 30°C. 4.5 gr of the desired product are thus obtained. Quantitative determination of the benzyl alcohol content is performed by gas chromatography following alkaline hydrolysis. The total ester group content is measured by the saponification method described on pages 169-172 of "Quantitative analysis via functional groups", fourth edition, John Wiley and Sons Publication.

The ester derivative thus prepared is solubilized to a concentration of 150 mg/ml in DMSO at a temperature of 30°C. The solubilized derivative is filtered through a 20 micron mesh and placed in an extrusion reactor connected to a film extruder with a thickness of <1mm. The product is extruded in a coagulation bath containing a solvent which enables the DMSO to be extracted from the product (e.g. ethanol), and the material from the film extruder is wound onto a series of rolls equipped with air fans to dry the membrane.

PRECLINICAL STUDIES

The following studies report results which show the usefulness of the products of the invention in preventing post-surgical adhesions and show the improved results for those products as compared to prior existing products.

Study 1

This study demonstrates the high incidence of surgical adhesion formation observed in a model of lesion induced in rat liver, established as the positive control to compare the preventive action of healthcare and surgical articles derived from HA, in adherence formation.

For these experiments, Sprague Dawley rats weighing between 275 and 300 gr were used. 21 animals were subjected to lesions.

Each animal underwent laparotomy by abdominal incision after anaesthetic with a dilution of Ketamine, 100 mg/Kg and Xylazine 11 mg/Kg, prepared in sterile conditions and injected by the intramuscular route.

The liver was located and exposed; an abrasion was produced on the lower lobe by applying slight pressure with a sterile tampon until blood was drawn. After hemostasis of the injured surface, the laparotomy was closed with a size 3.0 silk suture. The animals were sacrificed after 7 to 21 days.

Adhesion was assessed according to the ease with which the adjacent surfaces (upper and lower) of the lobe could be separated by surgical pincers, on the basis of the following scale:

0 no adhesion - the two surfaces can be separated

1 slight - moderate adhesion, the surfaces can be separated by pulling them apart with pincers;

2 notable adhesion between the two surfaces, any attempt to separate them causes the tissues to tear;

In this animal model, adherences which scored 2 were considered clinically significant.

In this positive control group (adherence formation) 17 animals out of 21 (80.9%) presented the formation of adherence with a score of 2.

<u>Study 2</u>

This study illustrates the significant reduction in the formation of adherences when a gel made of cross-linked hyaluronic acid (ACP) is used or a gauze based on HYAFF 11 (benzyl ester of HA) is used alone or in combination with hemostatic Surgicel™ and Heparin 50 IU/ml. The ACP gel was spread over the surface to be treated.

The surgical protocol described in Example 1 was used as an animal model to induce adhesion formation.

The significant reduction in adhesion formation between the two adjacent surfaces of the left lobe of the liver is illustrated in Table 1.

TABLE 1

MATERIAL	NUMBER OF ANIMALS	% OF SIGNIFICANT ADHESIONS (score 2)
HYAFF 11 woven tissue	6	50%
HYAFF 11 woven tissue + Surgicel™	6	16%
HYAFF 11 woven tissue + Surgicel™ + heparin	6	16%
HYAFF 11 woven tissue + heparin	6	33%
HYAFF 11 membrane (20-mm thick)	11	36%
HYAFF 11 non-woven tissue + Surgicel™	6	33%
ACP	24	20%

It is evident that the use of these slowly biodependable biomaterials as an impermeable barrier to inflammatory cells between two adjacent surfaces reduces the formation of adhesions, as compared to 80.9% of adhesion observed in the control group described in Example 1.

Study 3

This example shows the high incidence of surgical adhesion formation observed in a model of surgical lesion induced on the abdominal wall in rat to establish as a positive control and to compare that with the preventive action of healthcare articles comprised of HA derivatives of the invention (HYAFF 11 + a polypropylene mesh) in adherence formation.

A total of 24 animals (12 control, 12 test) underwent lesion.

Each animal underwent laparotomy by abdominal incision following anaesthesia with a dilution of Ketamine 100 mg/kg and Xylazine 11 mg/kg prepared in sterile conditions and injected by the intramuscular route.

The flap to the left of the incision was raised with two surgical pincers in order to expose the abdominal wall. An area of 1.5 cm x 1.5 cm of the peritoneal surface was removed with surgical scissors until exudate appeared, without removing the muscle bundle. In the control group, it was necessary to stitch a polypropylene mesh (measuring twice the area of the lesion) with a size 6.0 bioabsorbable Vycil suture over the injured surface in order to guarantee the tensile resistance of the abdominal wall. Before applying the material, the injured surface had to undergo thorough hemostasis.

After sacrifice at 14 days, the intermediate time of the range cited in Example 1, adherence was assessed according to the following scale:

0 absence of adhesion;

1 slight adhesion with no vascularization, can easily be separated;

2 moderate adhesion with no vascularization, can be pulled apart manually;

3 firm adherence, opaque and vascularized, difficult to separate, requiring the use of a scalpel;

4 very firm adherence, thick, opaque and vascularized, can only be cut apart with surgical scissors, with consequent destruction of tissues.

Adherences with a score of >2 were considered significant.

In the positive control group (adherence formation) 12 animals out of 12 (100%) presented adherence formation with a score of >2; whereas there was a

significant reduction in the incidence of adhesion formation between the abdominal wall and inner organs when utilizing the product of the invention, as shown in Table 2.

TABLE 2

MATERIAL	NUMBER OF ANIMALS	% OF SIGNIFICANT ADHESIONS (score 2)
HYAFF 11 + polypropylene mesh	12	25%
Control - polypropylene mesh	12	100%

It is evident that the use of the said HYAFF 11 material of the invention as a barrier (impermeable to inflammatory cells) between an injured, inside surface (abdominal wall) and the adjacent organs, reduces the formation of adhesions, as compared to 100% of adhesions in the control group treated with only a polypropylene mesh.

Study 4

This study illustrates the ability of auto-cross-linked hyaluronic acid (ACP) in the form of a gel and used as a coating to reduce surgical adhesion formation, in a model of lesion induced in the blind intestine of rat.

This type of lesion induces the formation of adhesions when treated with saline washing and hemostasis alone after surgery, as reported hereafter.

As in Example 1, Sprague Dawley rats weighing 275-300 gr were used. Each animal underwent laparotomy by abdominal incision after anaesthetic with a dilution of Ketamine, 100 mg/Kg and Xylazine 11 mg/Kg, prepared in sterile conditions and injected by the intramuscular route. The blind intestine was located and exposed. A thermal lesion was induced on the surface of the intestine with a solid body using a copper disc with a

diameter of 1 cm connected to a soldering appliance electronically set at a temperature of 69.5°C. This was left in contact with the intestinal surface for 15 seconds. A well-defined lesion with exudate was produced. After washing the injured area with saline and performing hemostasis with Surgicel™, the laparotomy was closed with a size 3.0 silk suture.

After sacrifice at 14 days, the intermediate time of the range cited in Example 1, adherence was assessed according to the following scale:

0 absence of adhesion;

1 slight adhesion with no vascularization, can easily be separated;

2 moderate adhesion with no vascularization, can be pulled apart manually;

3 firm adherence, opaque and vascularized, difficult to separate, requiring the use of a scalpel;

4 very firm adherence, thick, opaque and vascularized, can only be cut apart with surgical scissors, with consequent destruction of tissues.

In this animal model, adherences with a score of >2 were considered significant.

There is an evident reduction in the formation of adhesions when ACP gel is used as a barrier, as compared to the controls treated with saline washing and hemostasis alone (Table 3).

TABLE 3

MATERIAL	NUMBER OF ANIMALS	% of SIGNIFICANT ADHESIONS (score 2)
Control (saline + hemostasis)	17	70%
ACP	11	40%

It is evident that the use of said material as a barrier reduces the formation of adhesions, as compared

with the control treatment of saline washing and hemostasis alone.

Study 5 - Effect of Hyaluronic Acid Derivatives HYAFF-7 and HYAFF 11p75 in the Prevention of Postsurgical Adhesions in the Hepatic Lesion Model in Rat

Animal model:

Male, Harlan SD rat weighing 250 gr.

Type of lesion:

The abdominal area was thoroughly cleansed with an iodine solution, then a laparotomy of about 3 cm was performed to expose the liver. The lower right lobe of the liver was damaged by abrasion and a lesion produced with a sterile wooden spatula until blood was drawn.

Test materials:

Experiment 1: HYAFF 11p75, 75% partial benzyl ester of hyaluronic acid in the form of a gauze and a nonwoven tissue.

Experiment 2: HYAFF 7, total ethyl ester of hyaluronic acid in the form of a gauze and.a nonwoven tissue.

- Application of the material: after careful hemostasis with a conventional hemostatic, the test and control materials were placed between the lower (lesion area) and the upper hepatic lobes (adjacent surfaces) without the use of suture so as to form a barrier effect and prevent the formation of adhesions.

Assessments and observations:

Observations were made between seven and twenty-one days following surgery. The adhesions which had formed were assessed on the basis of the following visual score:

0 = Absence of adhesions

1 = Slight adhesions

2 = Notable presence of adhesions

Besides assessment by the adhesion score, the degree of inflammation was assessed by microscopic observation (tissue reaction to application of the material), staining the histological samples with hematoxylin/eosin and Mallory's triple stain.

Results

Experiment 1:

In Experiment 1, the materials based on HYAFF 11p75, partial benzyl ester of hyaluronic acid, were tested alone, in combination with Surgicel hemostatic and in combination with the hemostatic plus heparin saturation (1,000 Uml). These procedures are common practice in surgery.

Figure 1 is a graph showing the performance of the biomaterials when used alone. No effect on adhesion prevention was observed in the case of the biomaterials based on HYAFF 11p75 and Interceed, and even though the trend did seem better, albeit not significantly different, in the latter case, the hepatic lobes were completely adhered and a significant inflammatory reaction could be seen. The same was observed on histological observation of the biopsies, where a notable presence of inflammatory cells, neutrophils and macrophages, and mature collagenous fibers could be seen.

In Figures 2 and 3 the materials were used in combination with Surgicel and Surgicel + heparin. The trend observed in Figure 1 was confirmed by the materials based on HYAFF 11p75, while Interceed saturated with heparin seemed to give better effects. This situation was confirmed by the histological observations.

In conclusion, the materials based on HYAFF 11p75 cannot be used in the prevention of postsurgical adhesions, as the inflammatory effect is probably due to

the release of oligomers of low-molecular-weight hyaluronic acid, in view of the extremely brief degradation times of the products.

Experiment 2

In Experiment 2, the biomaterials based on HYAFF 7, ethyl ester of hyaluronic acid, were tested in combination with Surgicel and Surgicel + heparin. In neither case was an effect on the prevention of postsurgical adhesions to be observed. Interceed used with Surgicel + heparin seemed to have the more positive effect (Fig. 4).

Microscopic observation confirmed these data and revealed a notable quantity of inflammatory cells and collagen fibers in the case of treatment with HYAFF 7. In this case, as in the last, the biomaterials based on HYAFF 7 cannot be used in the prevention of postsurgical adhesions, as it is likely that there is a progressive release of ethanol into the organism.

Study 6 - Efficacy of HYAFF 11-Based Biomaterials in the Prevention of Postsurgical Adhesions in Two Different Models of Lesion Induced in Animals: 1) Intrahepatic Abrasion in Rat; 2) Lesion of the Abdominal Wall in Rat

Animal Model 1

Once the abdominal area had been disinfected with iodine and ethanol, a medial incision was made to expose the liver.

In this animal model, the inner surface of the lower hepatic lobe was scraped until exudate began to emerge. The abrasion received careful hemostasis with Tabotamp (Ethicon) and the material was left on the damaged surface without the aid of suture because of the product's highly mucoadhesive characteristics.

Two HYAFF 11-based products were tested, both commercial versions of a 20 μm thick, continuous membrane, called **Transprocess** and **Hyalobarrier 20**. Macroscopic assessment was made 14 days after surgery using a score system described above to define the

adhesions. A further assessment was made of the percentage of animals with adhesion score=2 (significant adhesion).

Results

Figure 5 is a graph representation of the adhesion scores obtained in the experiment. Hyalobarrier 20 reduces the incidence of adhesion formation compared to the non-treated controls and to the two treatments with high- and low-molecular weight hyaluronic acid. A similar trend was recorded, albeit without any statistically significant differences, in the case of the other HYAFF 11-based material, Transprocess. Figure 6 shows the percentages of cases of adhesion score=2 in each treatment group (surgically significant adhesion). The tendency revealed by the previous graph (Fig. 1) was confirmed in this case too, with a reduction in adhesion scores=2 (percentage of less than 50% for the Hyalobarrier 20 and Transprocess treatments.

Animal Model 2

Once the abdominal area had been disinfected with iodine and ethanol, a median laparotomy of about 5 cm in length was made to expose the abdominal wall and peritoneum.

An incision of 2 cm x 2 cm was made with a scalpel and then the peritoneum and the muscular layer were removed. In this type of operation, it is necessary to suture to the damaged area a material which favors tissue growth while guaranteeing adequate tensile strength, in order to avoid the collapse of the peritoneal wall. Generally, nondegradable materials with a polymeric matrix are used, such as meshes of polypropylene, polyester or expanded polytetrafluoroethylene. The use of such materials alone, however, is not sufficient to avoid the formation

of adhesions to the intestinal loops, with consequent intestinal obstruction and chronic pain.

Macroscopic assessments were made 14 days after surgery by applying adhesion scores running from 0 to 4. A further assessment was made of the percentage of animals with an adhesion score of >2 (significant adhesions).

Results:

This experiment demonstrates that a coating of HYAFF 11 on a synthetic Prolene mesh (polypropylene mesh, widely used in abdominal surgery) and a sheet of HYAFF 11 on a Prolene mesh attached by suture can reduce the formation of postsurgical adhesions. Fig. 7 shows that the combined product called Hyalobarrier Plus (HYAFF 11 spread and coagulated on prolene) and the Hyalobarrier film suture on prolene significantly reduce adhesions compared to prolene mesh alone. Fig. 8 confirms this trend, with a lower percentage of adhesions >2 (significant adhesions) following treatment with HYAFF 11 than was observed after treatment with prolene mesh alone.

Study 7 - Effects of ACP Gel Biomaterials on the Prevention of Postsurgical Adhesions Formation at 14 Days in a Rat Liver Injury Model and in a Rat Intestine Injury Model

The purpose of this study was to evaluate the efficacy of ACP gel-based biomaterials, to reduce or prevent postoperative adhesions formation. The performances of the test materials were assessed in comparison to the hyaluronic acid high molecular weight and to the commercially available biomaterials, Oxidized Regenerated Cellulose (TC 7 Interceed*) used in abdominopelvic and gynecological surgery to prevent adhesion formation.

A rat liver lesion model and a rat intestine burn model were used since they are characterized models of

experimental adhesion induction. The effects of the test and control materials on the prevention of postsurgical adhesion were evaluated by gross observation of the site of lesion applying an adhesion score.

A rat liver injury model (Experiment 1) and a rat intestine injury model (Experiment 2) were used since they are standardized and reproducible models of experimental adhesion induction. ACP based biomaterials were used after injury as a barrier between adjacent surfaces of the epatic lobe and internal organs.

In both experiments the efficacy of ACP gels was evaluated for their ability to prevent or reduce adhesion formation in comparison with TC7 Interceed, an absorbable Oxidized Cellulose adhesion barrier widely used in clinical practice, a copolymeric solution "Thermogel", a solution of High Molecular Weight Hyaluronic Acid and a group of untreated animals (sham operated).

Start date:

<u>Experiment 1 - Rat Liver Abrasion</u>

Tested Materials:

	1	2
PRODUCT CODE	SMK 0002	SMK 0002
COMMON NAME	ACP Gel	ACP Gel
COMMERCIAL NAME	Hyalogel Barrier	Hyalogel Barrier
SUPPLIER	FAB	FAB
LOT NUMBER	101/96	104/96
EXPIRY DATE	20-02-96	20-02-96
STORAGE	below 30°C	below 30°C
PRECAUTIONS	none	none

ACP gels were suspended in water at the concentration of 60 mg/ml. The test materials were supplied sterile by autoclave and in 5 ml syringe and manipulated in sterile conditions. The ACP gels were

applied as to coat the abraded liver lobe surfaces after hemostasis with Tabotamp®. Each animal received an amount sufficient to completely coat the injured area (about 2 ml) by single administration at time of surgery.

Control Materials:

	1	2	3
TRADE NAME	TC7 Interceed*	HYAL	Thermogel
MANUFACTURER/ SUPPLIER	Johnson & Johnson Patient Care, New Brunswick, NJ	FAB	BASF Pharma
DESCRIPTION	Oxidized regenerated cellulose barrier	Hyaluronic Acid (M.W. 800,000)	Pluronic Acid
LOT NUMBER	2710TCM	0108 st	1/95
EXPIRY DATE	11-97	05-97	--
STORAGE	below 30°	below 30	below 8°C
PRECAUTIONS	none	none	

Interceed was cut under sterile handling conditions, it was used alone and saturated in Heparin (500 U/ml), then applied so as to keep separated the two adjacent surfaces of the hepatic lobes to a size exceeding the borders of the injured area by several mm. HYAL, Hyaluronic Acid High Molecular Weight (solubilized in water at the concentration of 10 mg/ml) and Thermogel were purchase in sterile syringe.

Interceed was applied by direct application without surgical suture. HYAL, Hyaluronic Acid and Thermogel were applied to the injured surface (coating) with a syringe after hemostasis. Each animal received an amount sufficient to completely coat or cover the injured area by single administration at time of surgery.

EXPERIMENTAL DESIGN

Sprague Dawley rats (275-300 g) were utilized for this experiment. From the experience gained from previous experiments, a period of 14 days was considered an adequate time point to evaluate adhesion formation in these animal models. Given the number of animals required for this study, animals were prepared on successive days.

A total number of 78 animals were used according to the following scheme:

GROUP	TREATMENT	NUMBER OF ANIMALS
Sham Operated	Untreated	12
Controls	TC7 Interceed™Alone	12
Controls	TC7 Interceed™+ Heparin	6
Controls	HYAL®	12
Controls	Thermogel	12
Treated	ACP 5% (batch 101/96)	12
Treated	ACP 5% (batch 104/96)	12

Preparation of the animals:

Animals were anesthetized by i.m. Ketamine (Gellini Pharmaceutical)/Xylazine (Bayer) injection, shaved and then disinfected with iodine solution and ethanol. Following laparotomy on the left side, the left lobe of the liver was reflected upwards and the inner surfaces of the left and medial lobes of the liver were abraded by gentle rubbing with a wooden applicator until evidence of bleeding or serous exudate was obtained.

Administration of materials:

After hemostasis obtained with Surgicel® or Tabotamp™, test and control materials were placed between the surfaces of the two lobes so as to cover the

entire abraded area and to create a barrier between the lobes.

The surgical site was closed in two layers with 3.0 silk sutures.

At the end of surgery an antibiotic (Procacillina sub-cutaneous 30,000 I.U./rat) and an analgesic (Temgesic I.M. 0.05 mg/Kg) were administered for 4 days.

<u>Adhesion grade</u>:

14 days after surgery, animals were euthanized by CO_2.

The adhesion grade was evaluated by gross observation. The following adhesion score was applied:

0 = No adhesion

1 = Low to moderate adhesion. The two epatic lobes were surgically separated by mechanical traction by forceps.

2 = Marked adhesion, the two epatic lobes were completely sticked, any attempt to separation caused the breaking of the tissue.

The resorbability of the materials was evaluated by visual assessment of the presence of the materials; furthermore, the site of treatment was photographed.

After gross observations were made, the entire liver was surgically removed and placed in 10% buffered formalin for 48 hours. After fixation, a 2.0 mm cross-section including the abraded area, was removed from the liver by using a dissecting blade. The specimens so obtained were subjected to histological analysis.

Analysis of the Tissue:

<u>Histological analysis</u>:

Specimens were fixed in neutral buffered formalin 10% and subsequently dehydrated and embedded in paraffin by standard techniques; 8 μm section was stained with Masson's Trichnome (for tissue inflammatory reaction)

and Toluidine Blue if necessary (for material remnants presence).

EXPERIMENT 2 - Rat Intestine Burn

Tested Materials:

	1	2
PRODUCT CODE	SMK 0002	SMK 0002
COMMON NAME	ACP 5% High Mol. Weight	ACP 5%
COMMERCIAL NAME	Hyalogel Barrier	Hyalogel Barrier
SUPPLIER	FAB	FAB
LOT NUMBER	3/94	ACP 5% (batch 101/94
EXPIRY DATE	07/95	07/95
STORAGE	below 30°C	below 30°C
PRECAUTIONS	none	none

ACP 5% gels High M.W. batch 3/94 was suspended in water at the concentration of 20 mg/ml, ACP 5% batch 101/94 was suspended at the concentration of 50 mg/ml. All the test materials were supplied sterile by autoclave and in 5 ml syringe and manipulated in sterile conditions. ACP gels were applied as to as to coat the burnt intestinal surfaces after hemostasis with Tabotamp®. Each animal received an amount sufficient to completely coat the injured area (about 2 ml) in a single dose administration at time of surgery.

Control Materials:

	1	2	3
TRADE NAME	TC7 Interceed*	HYAL	Thermogel
MANUFACTURER/ SUPPLIER	Johnson & Johnson Patient Care, New Brunswick, NJ	FAB	BASF Pharma
DESCRIPTION	Oxidized regenerated cellulose barrier	Hyaluronic Acid (M.W. 1,200,000)	Pluronic Acid
LOT NUMBER	2710TCM	0108 st	1/94
EXPIRY DATE	11-97	05-97	--
STORAGE	below 30°	below 30	below 8°C
PRECAUTIONS	none	none	

Interceed was cut under sterile handling conditions, it was used alone and saturated in Heparin (500 U/ml), then applied so as to keep separated the two adjacent surfaces of the hepatic lobes to a size exceeding the borders of the injured area by several mm. HYAL, Hyaluronic Acid High Molecular Weight (solubilized in water at the concentration of 10 mg/ml) and Thermogel were purchased in sterile syringe.

Interceed was applied by direct application without surgical suture. Hyaluronic Acid and Thermogel were applied to the injured surface (coating) with a syringe after hemostasis. Each animal received an amount sufficient to completely coat or cover the injured area by single dose administration at time of surgery.

EXPERIMENTAL DESIGN

Sprague Dawley rats (275-300 g) were utilized for this experiment. From the experience gained from previous experiments, a period of 14 days was considered an adequate time point to evaluate adhesion formation in this animal model.

Given the number of animals required for this study, animals were prepared on successive days.

A total number of 59 animals were used according with the following scheme:

Experiment 2:

GROUP	TREATMENT	NUMBER OF ANIMALS
Sham Operated	Untreated	10
Control	TC7 Interceed™	6
Control	Hyaluronic Acid (M.W. 1 200 000)	12
Control	Thermogel	13
Treated	ACP 5% (batch 101/94)	12
Treated	ACP 5% High Mol. weight (batch 3/94)	6

Preparation of the animals:

Animals were anaesthetized by i.m. Ketamine (Gellini Pharmaceutical)/Xylazine (Bayer) injection, shaved and then disinfected with iodine solution and ethanol. A midline abdominal incision was made through the skin and muscle tissue so as to expose the intestine. Burn was produced by application to the cecum surface of an electronically controlled heated copper disk (1 cm diameter) using a standard pressure for 15 sec. at 158°F. (69.3°C).

Administration of materials:

After hemostasis obtained with Surgicel® or Tabotamp™, test and control materials were placed on the intestine surface without suture so as to cover the entire burned area and to create a barrier between the peritoneum and internal organs.

The musculo peritoneal layer were closed with continuous 3-0 silk sutures, the cutaneous layer with skin staples and 3-0 silk interrupt suture.

At the end of surgery an antibiotic (Procacillina sub-cutaneous 30,000 I.U./rat) and an analgesic (Temgesic I.M. 0.05 mg/Kg) were administered for 4 days.

Observations and Determinations

Adhesion grade:

14 days after surgery, animals were euthanized by CO_2.

The adhesion grade was evaluated by gross observation. The following adhesion score was applied:

0 = No adhesion

1 = Low, avascular, easily dissected

2 = Moderate, avascular, continuous, manual dissected

3 = Opaque, vascular, difficult to section requiring scalpel separation

4 = Dense, opaque, vascular, dissected only with surgical scissors and tissue damage.

The resorbability of the materials was evaluated by visual assessment of the presence of the materials; furthermore, the site of treatment was photographed.

Results

Experiment 1

One animal died during anaesthesia administration, the placement of the biomaterials was easily accomplished. The materials were noted to adhere to the tissue of the lower epatic lobe. No clinical signal of disease or suffering was noted after surgery in the treated animals with ACP.

Two animals treated with Hyaluronic Acid died two days after surgery. Necroscopic examination showed internal hemorrhage.

Evaluation of adhesion formation: The adhesions formed between the two adjacent surfaces of the epatic lobe following tissue damage, were evaluated at 14 days. All treatments were degradated at the time of

observation; the adhesion score (Fig. 9) in the animals treated with ACP 5% (batch 104/96) biomaterial was significantly lower than all control materials and untreated control. In the ACP 5% (batch 101/96) treatment, the reduction of adhesion was superior than TC 7 Interceed saturated with heparin but no statistical differences were noted; nevertheless both treatments showed significant differences ($p<0.05$) if compared to the controls and untreated.

Histomorphological observation: At microscopic examination, 14 days after surgery, the ACP treatments were found to be highly biocompatible and a very low inflammatory reaction was observed, in particular, few inflammatory cells, as neutrophils and giant cells, were present, no migration or enhancements of these cells inside the gap between the two lobes was noted, TC 7 Interceed* showed tissue reaction consequently in many cases the epatic surfaces were partially sticked; the scope observation emphasized the presence of organized collagen fibrils, the inflammatory reaction seems to decrease if this treatment is saturated with heparin solution. In the majority of the slides the biomaterials appeared completely biodegradated. The untreated control gave moderate-high inflammatory reaction.

Experiment 2

A total number of 2 animals died during anaesthesia administration, the placement of the biomaterials was easily accomplished and they did not move after placement. No clinical signal of disease or suffering was noted after surgery in the ACP treatment group animals.

Four animals treated with Hyaluronic Acid mol. weight $1.2 \cdot 10^6$ and three animals with Thermogel died between two and five days after surgery. Necroscopic examination showed internal hemorrhage in all animals.

Evaluation of adhesion formation: In this experiment, 14 days after surgery (Fig. 10), the ACP 5% gels 101/94 and 3/94, showed a reduction of postsurgical adhesion formations if compared to Hyaluronic Acid mol. weight $1.2 \cdot 10^6$ and untreated control, the performance of ACP gels was comparable to that of an Interceed Barrier, heparin saturated, statistical difference was found between these treatments and untreated control groups ($P<0.05$); all treatments and controls were completely absorbed.

Histomorphological observation: At scope observation at 14 days, the ACP treatments showed a low tissue inflammatory reaction, the thickness of granulation tissue was very low and no unfavorable reaction on the intestine as adhesion to the adjacent peritoneal surface were noted. The fibrils collagens begin to organize and healing process was completed. An inflammatory reaction was observed in hyaluronic acid treatments with considerable presence of collagen fibers that induce adhesion; a thick granulation tissue was seen. The same histomorphological appearance was noted in the untreated control.

Discussion

Adhesions formation are among the leading cause of postoperative morbidity following abdominopelvic surgery, frequently leading to small bowel obstruction and other important pathologies. When the pelvic viscera are involved, these adhesions have the potential to impair physiologic function and result in infertility. The mechanism of postsurgical adhesion formation and reformation remain poorly understood. The experimental evidence suggest that adhesions form between two surgically traumatized surfaces in natural apposition during the healing process because it is more efficient to combine two site of tissue repair into a

single healing site, resulting in coalescing adhesions between two adjacent surfaces.

In this screening, it was found that the use of a conventional surgical hemostatic agent (Surgicel®) after surgery and the successive placement of a biodegradable hyaluronic acid derivative barrier, prevents the formation of adhesions. In addition, the materials may be used in conjunction with fibrinolytic agents. The adhesion reduction compared favorably with that of oxidized regenerated cellulose TC 7 Interceed*.

HYAFF® 11 biomaterials showed good biocompatibility and very low inflammation. The rate of degradation of the FAB biomaterials tested was different and depend from the different physical form of the treatment; HYAFF 11® biomaterials persisted several weeks. The range of controlled degradation rates which may be achieved with these Hyaluronic Acid derivatives may be usefully exploited for the prevention of postsurgical adhesions in different anatomical sites and applications, e.g. gynecological or abdominopelvic areas.

The results suggest that hyaluronic acid derivatives (HYAFF® 11 gauze and membranes) have a role in the prevention of adhesion formation following surgery.

The invention being thus described, it will be obvious that the same may be varied in many ways. Such variations are not to be regarded as a departure from the spirit and scope of the invention, and all such modifications as would be obvious to one skilled in the art are intended to be included within the scope of the following claims.

Claims

1. A composite biomaterial for preventing surgical adhesions of tissue comprised of at least one hyaluronic acid derivative selected from the group consisting of:

(a) a benzyl ester of hyaluronic acid wherein 75 to 100% of the carboxyl groups of hyaluronic acid are esterified with a benzyl radical and up to 25% of the carboxyl groups are esterified with the alkyl radial of a C_{10} to C_{20} aliphatic alcohol, with the proviso that at least 80% of the carboxyl groups are esterified; and

(b) an auto-crosslinked derivative of hyaluronic acid wherein 0.5 to 20% of the carboxyl group of hyaluronic acid are cross-linked to the hydroxyl group of the same or different hyaluronic acid molecule.

2. The composite biomaterial according to claim 1, wherein said derivative is the total benzyl ester in which all of the carboxyl groups of hyaluronic acid are esterified with a benzyl group.

3. The composite biomaterial according to claim 1, wherein said derivative is a benzyl ester wherein 80% of the carboxyl groups are esterified with a benzyl group.

4. The composite material according to claim 1, wherein said derivative is a benzyl ester wherein 75% of the carboxyl groups are esterified with a benzyl group and the remaining 25% carboxyl groups are esterified with the aliphatic residue of a C_{10-20} aliphatic alcohol.

5. The composite material according to claim 4, wherein said alcohol is stearyl or palmitic alcohol.

6. The composite material according to claim 1, wherein said auto-crosslinked derivative has 4.5 to 5.0% of the carboxyl groups of the hyaluronic acid molecule cross-linked.

7. The composite material according to any one of claims 1-6 which further comprises a non-biodegradable synthetic polymer.

8. The composite material according to claim 7, wherein said synthetic polymer is a member selected from the group consisting of polypropylene, polyethylene, polyester and polytetrafluoroethylene.

9. The composite material according to any one of claims 1-8 in the form of a membrane, a mesh or a woven or non-woven tissue.

10. The composite biomaterial according to claims 1 or 6 in the form of a gel.

11. Use of a biomaterial for preventing surgical adhesions of tissue, wherein said biomaterial is comprised of at least one hyaluronic acid derivative selected from the group consisting of:

(a) a benzyl ester of hyaluronic acid wherein 75 to 100% of the carboxyl groups of hyaluronic acid are esterified with a benzyl radical and up to

25% of the carboxyl groups are esterified with the alkyl radical of a C_{10} to C_{20} aliphatic alcohol, with the proviso that at least 80% of the carboxyl groups are esterified; and

(b) an auto-crosslinked derivative of hyaluronic acid wherein 0.5 to 20% of the carboxyl group of hyaluronic acid are cross-linked to the hydroxyl group of the same or different hyaluronic acid molecule.

12. Use according to claim 11, wherein said derivative is the total benzyl ester in which all of the carboxyl groups of hyaluronic acid are esterified with a benzyl group.

13. Use according to claim 11, wherein said derivative is a benzyl ester wherein 80% of the carboxyl groups are esterified with a benzyl group.

14. Use according to claim 11, wherein said derivative is a benzyl ester wherein 75% of the carboxyl groups are esterified with a benzyl group and the remaining 25% carboxyl groups are esterified with the aliphatic residue of a C_{10-20} aliphatic alcohol.

15. Use according to claim 14, wherein said alcohol is stearyl or palmitic alcohol.

16. Use according to claim 11, wherein said auto-crosslinked derivative has 4.5 to 5.0% of the carboxyl groups of the hyaluronic acid molecule cross-linked.

17. Use according to any one of claims 11-16, which further comprises a non-biodegradable synthetic polymer.

18. Use according to claim 17, wherein said synthetic polymer is a member selected from the group consisting of polypropylene, polyethylene, polyester and polytetrafluoroethylene.

19. Use according to any one of claims 11-18, wherein said biomaterial is in the form of a membrane, a mesh or a woven or non-woven tissue.

20. A method for preventing surgical adhesions of tissue which comprises applying to tissue involved in surgery a biomaterial comprised of at least one hyaluronic acid derivative related from the group consisting of:

(a) a benzyl ester of hyaluronic acid wherein 75 to 100% of the carboxyl groups of hyaluronic acid are esterified with a benzyl radical and up to 25% of the carboxyl groups are esterified with the alkyl radical of a C_{10} to C_{20} aliphatic alcohol, with the proviso that at least 80% of the carboxyl groups are esterified; and

(b) an auto-crosslinked derivative of hyaluronic acid wherein 0.5 to 20% of the carboxyl group of hyaluronic acid are cross-linked to the hydroxyl group of the same or different hyaluronic acid molecule.

21. The method according to claim 20, wherein said derivative is the total benzyl ester in which all of the carboxyl groups of hyaluronic acid are esterified with a benzyl group.

22. The method according to claim 20, wherein said derivative is a benzyl ester wherein 80% of the carboxyl groups are esterified with a benzyl group.

23. The method according to claim 20, wherein said derivative is a benzyl ester wherein 75% of the carboxyl groups are esterified with a benzyl group and the remaining 25% carboxyl groups are esterified with the aliphatic residue of a C_{10-20} aliphatic alcohol.

24. The method according to claim 23, wherein said alcohol is stearyl or palmitic alcohol.

25. The method according to claim 20, wherein said auto-crosslinked derivative has 4.5 to 5.0% of the carboxyl groups of the hyaluronic acid molecule cross-linked.

26. The method according to claims 20-25, which further comprises a non-biodegradable synthetic polymer.

27. The method according to claim 26, wherein said synthetic polymer is a member selected from the group consisting of polypropylene, polyethylene, polyester and polytetrafluoroethylene.

28. The method according to claims 20-27, wherein said biomaterial is in the form of a membrane, a mesh or a woven or non-woven tissue.

FIG. 1

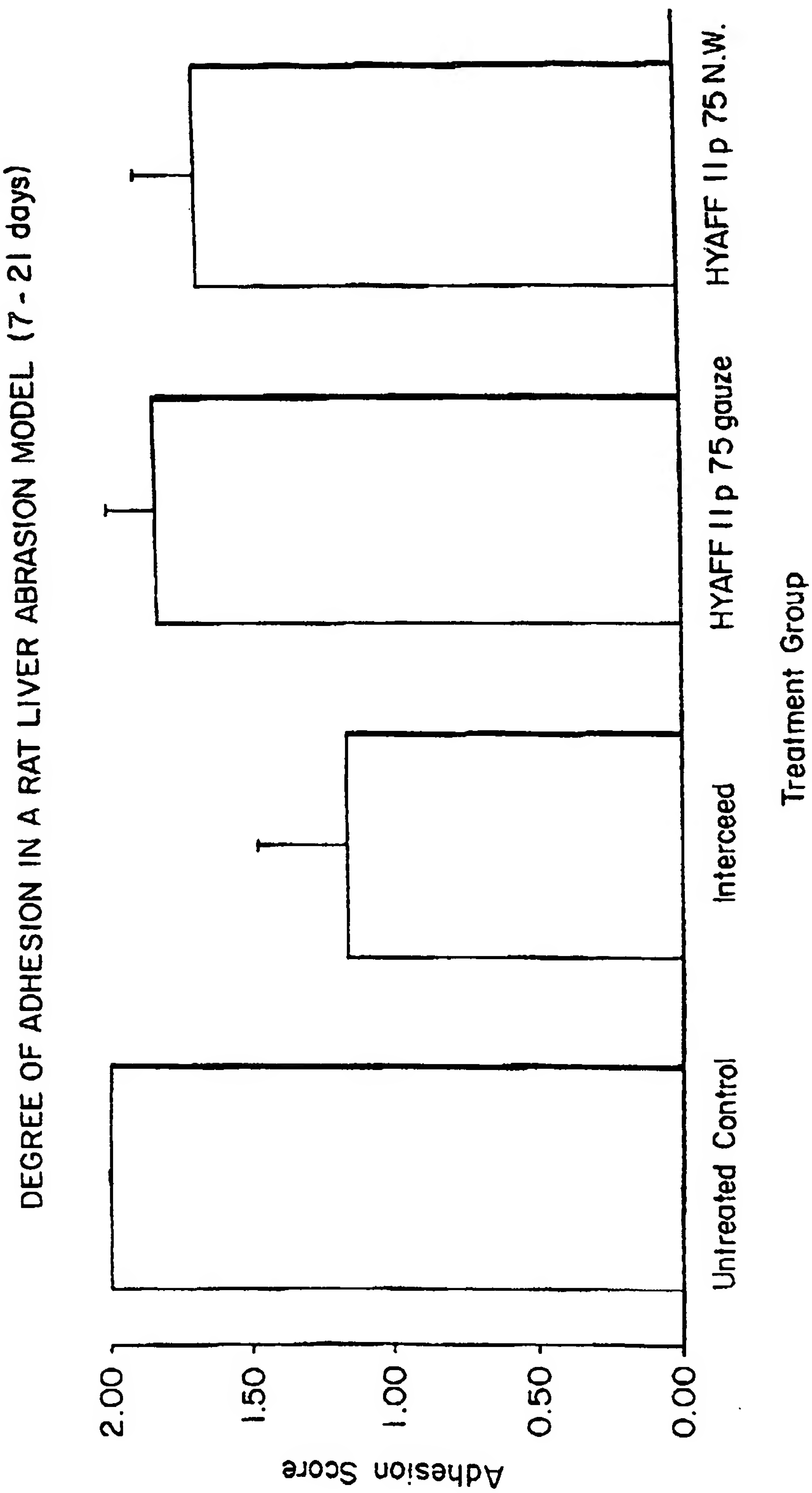

DEGREE OF ADHESION IN A RAT LIVER ABRASION MODEL (7 - 21 days)
Adhesion Score
2.00
1.50
1.00
0.50
0.00
Untreated Control
Interceed
HYAFF 11 p 75 gauze
HYAFF 11 p 75 N.W.
Treatment Group

FIG. 2

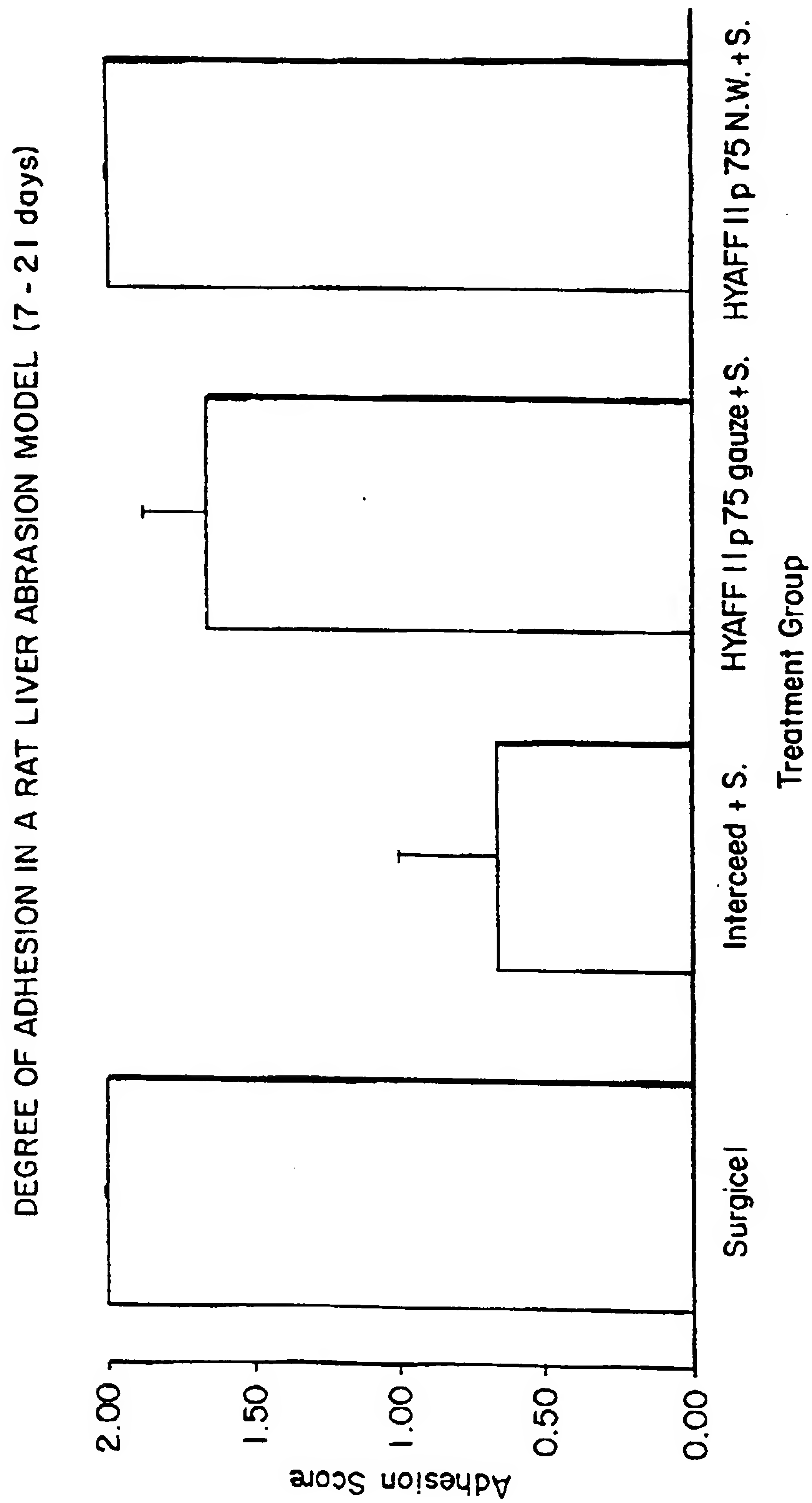

DEGREE OF ADHESION IN A RAT LIVER ABRASION MODEL (7 - 21 days)
Adhesion Score
2.00
1.50
1.00
0.50
0.00
Surgicel
Interceed + S.
HYAFF 11 p 75 gauze + S.
HYAFF 11 p 75 N.W. + S.
Treatment Group

Fig. 3

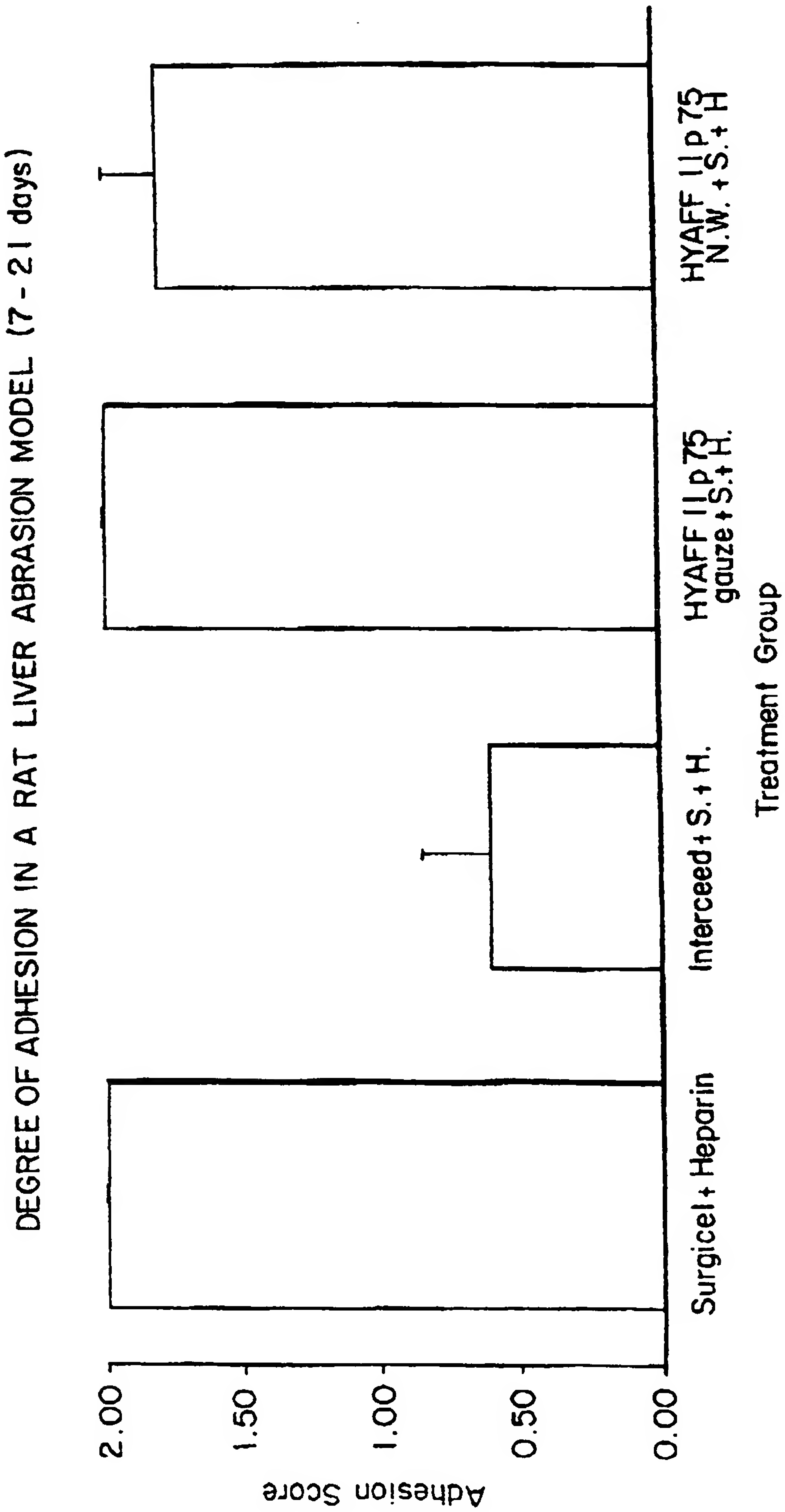

DEGREE OF ADHESION IN A RAT LIVER ABRASION MODEL (7 - 21 days)
Adhesion Score
2.00
1.50
1.00
0.50
0.00
Surgicel + Heparin
Interceed + S. + H.
HYAFF 11 p 75 gauze + S. + H.
HYAFF 11 p 75 N.W. + S. + H
Treatment Group

FIG. 4

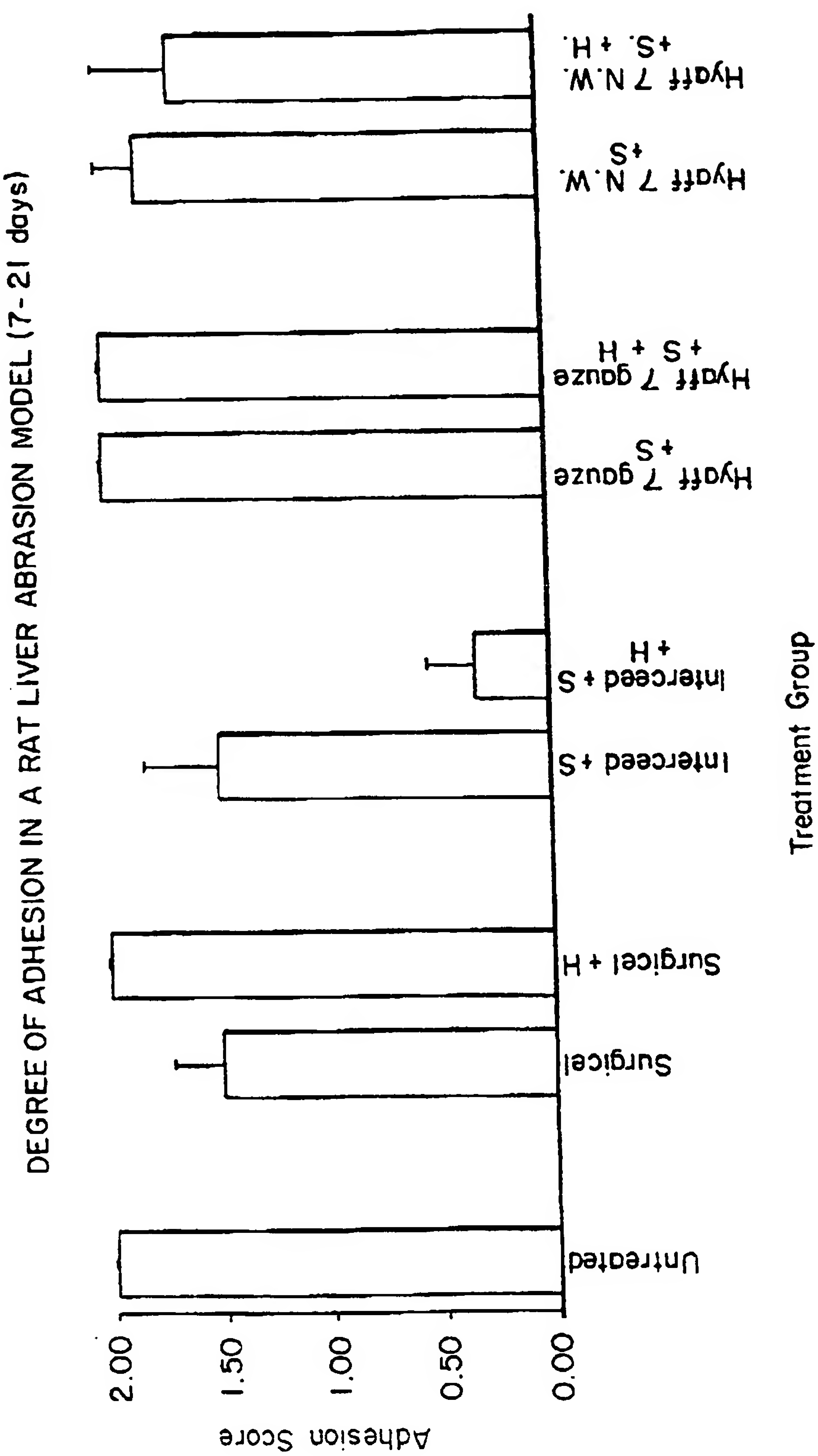

DEGREE OF ADHESION IN A RAT LIVER ABRASION MODEL (7-21 days)
Adhesion Score
2.00
1.50
1.00
0.50
0.00
Untreated
Surgicel
Surgicel + H
Interceed +S
Interceed + S + H
Hyaff 7 gauze +S
Hyaff 7 gauze +S + H
Hyaff 7 N.W. +S
Hyaff 7 N.W. +S. + H.
Treatment Group

FIG. 5

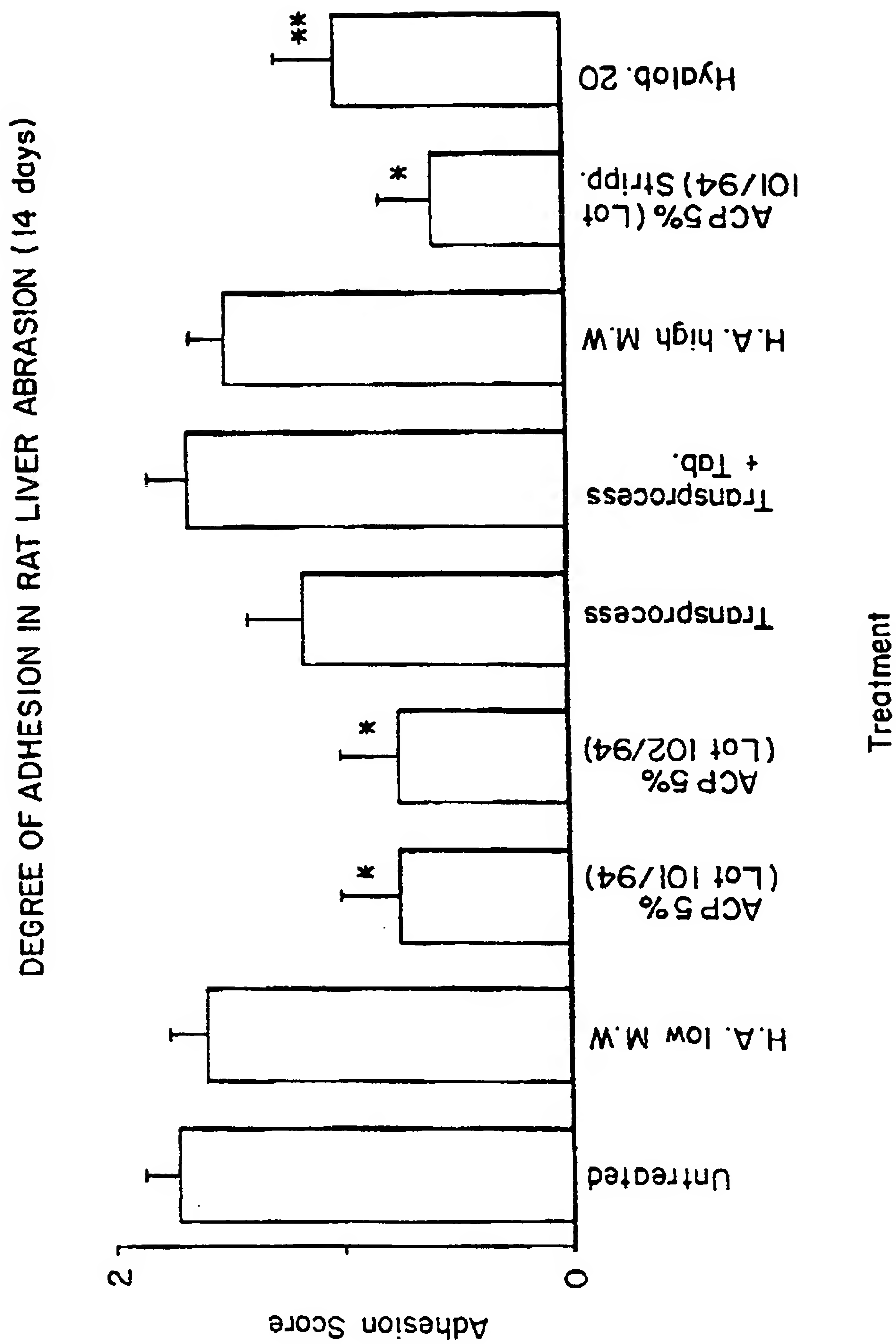

DEGREE OF ADHESION IN RAT LIVER ABRASION (14 days)
Adhesion Score
2
0
Untreated
H.A. low M.W
ACP 5% (Lot 101/94)
ACP 5% (Lot 102/94)
Transprocess
Transprocess + Tab.
H.A. high M.W
ACP 5% (Lot 101/94) Stripp.
Hyalob. 20
*
**
Treatment

FIG. 6

POSTSURGICAL ADHESION PREVENTION: % ANIMALS WITH ADHESIONS = 2* IN RAT LIVER ABRASION MODEL (14 days)

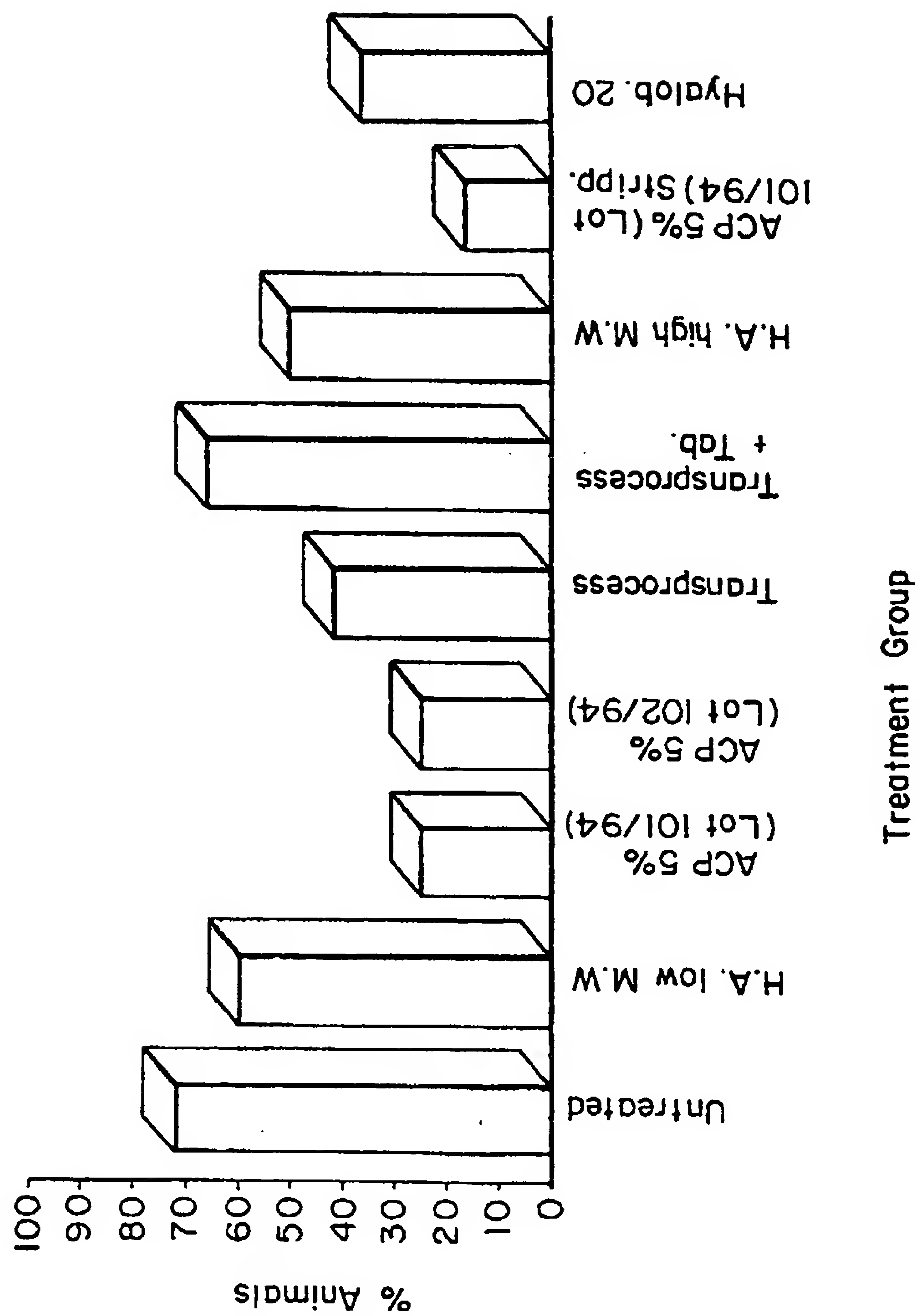


FIG. 7

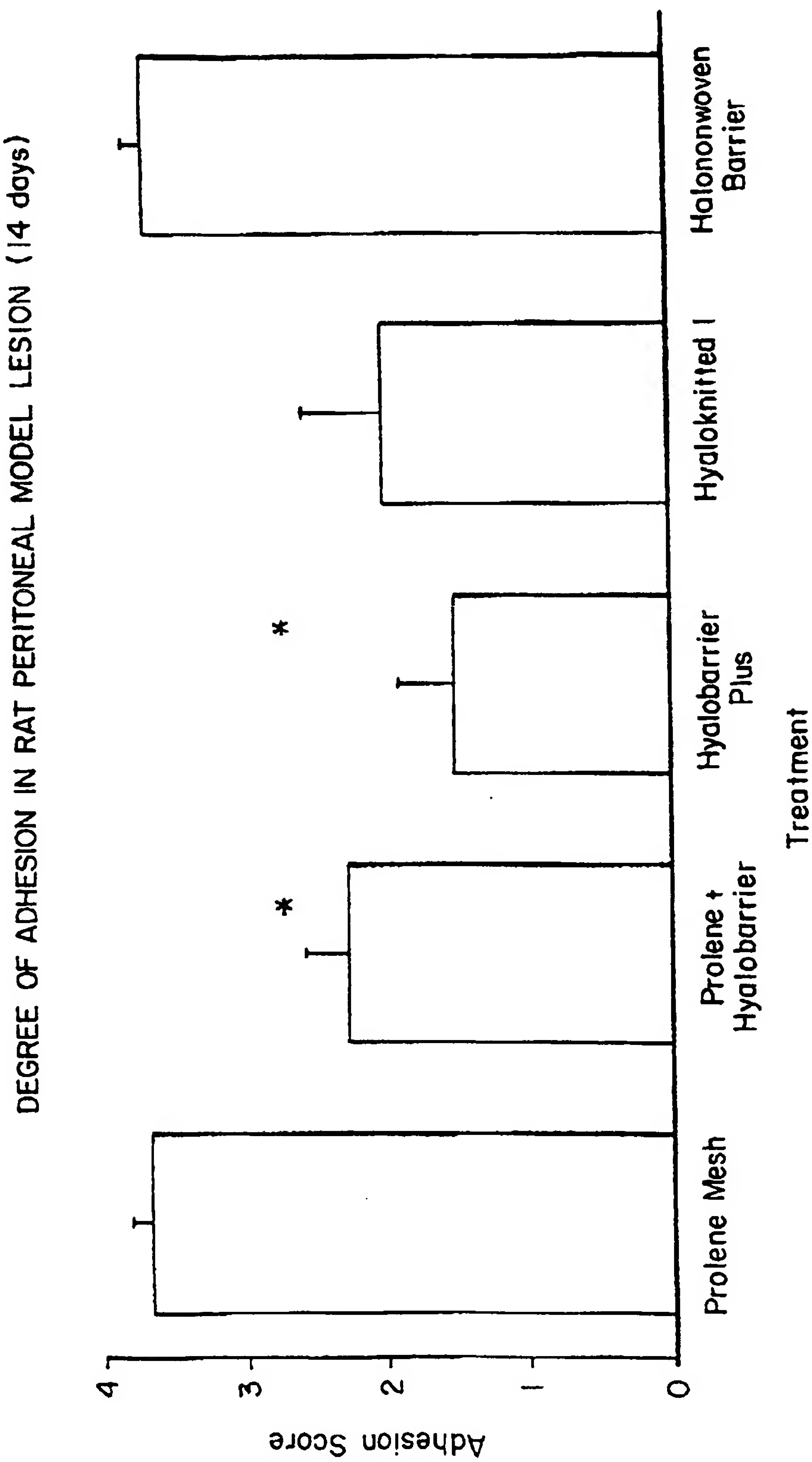

DEGREE OF ADHESION IN RAT PERITONEAL MODEL LESION (14 days)
Adhesion Score
0
1
2
3
4
Prolene Mesh
Prolene + Hyalobarrier
Hyalobarrier Plus
Hyaloknitted I
Halononwoven Barrier
Treatment
*
*

FIG. 8

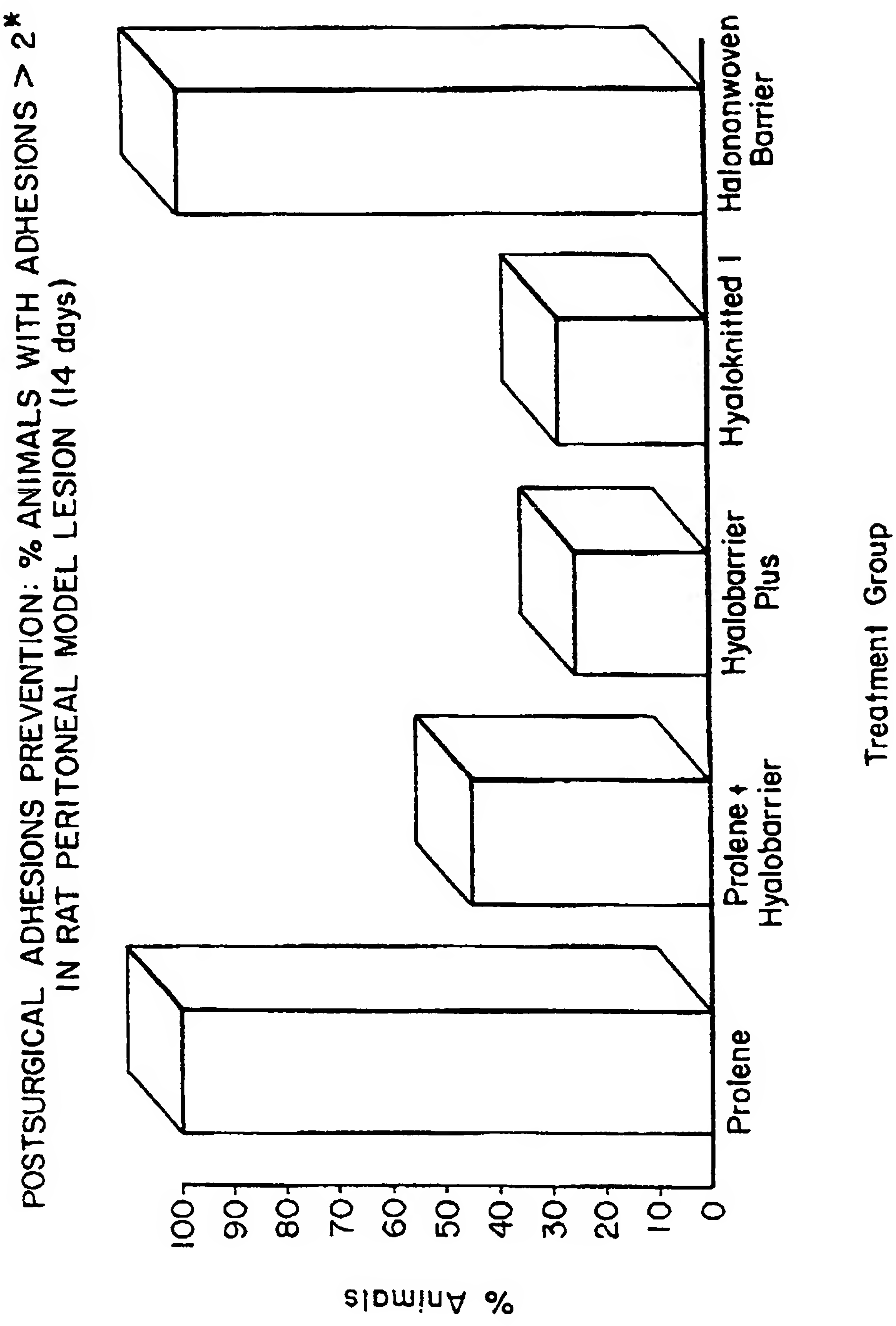

POSTSURGICAL ADHESIONS PREVENTION: % ANIMALS WITH ADHESIONS > 2*
IN RAT PERITONEAL MODEL LESION (14 days)
% Animals
100
90
80
70
60
50
40
30
20
10
0
Prolene
Prolene + Hyalobarrier
Hyalobarrier Plus
Hyaloknitted I
Halononwoven Barrier
Treatment Group

FIG. 9

POSTSURGICAL ADHESION PREVENTION: DEGREE OF ADHESION IN A RAT LIVER ABRASION MODEL (14 days)

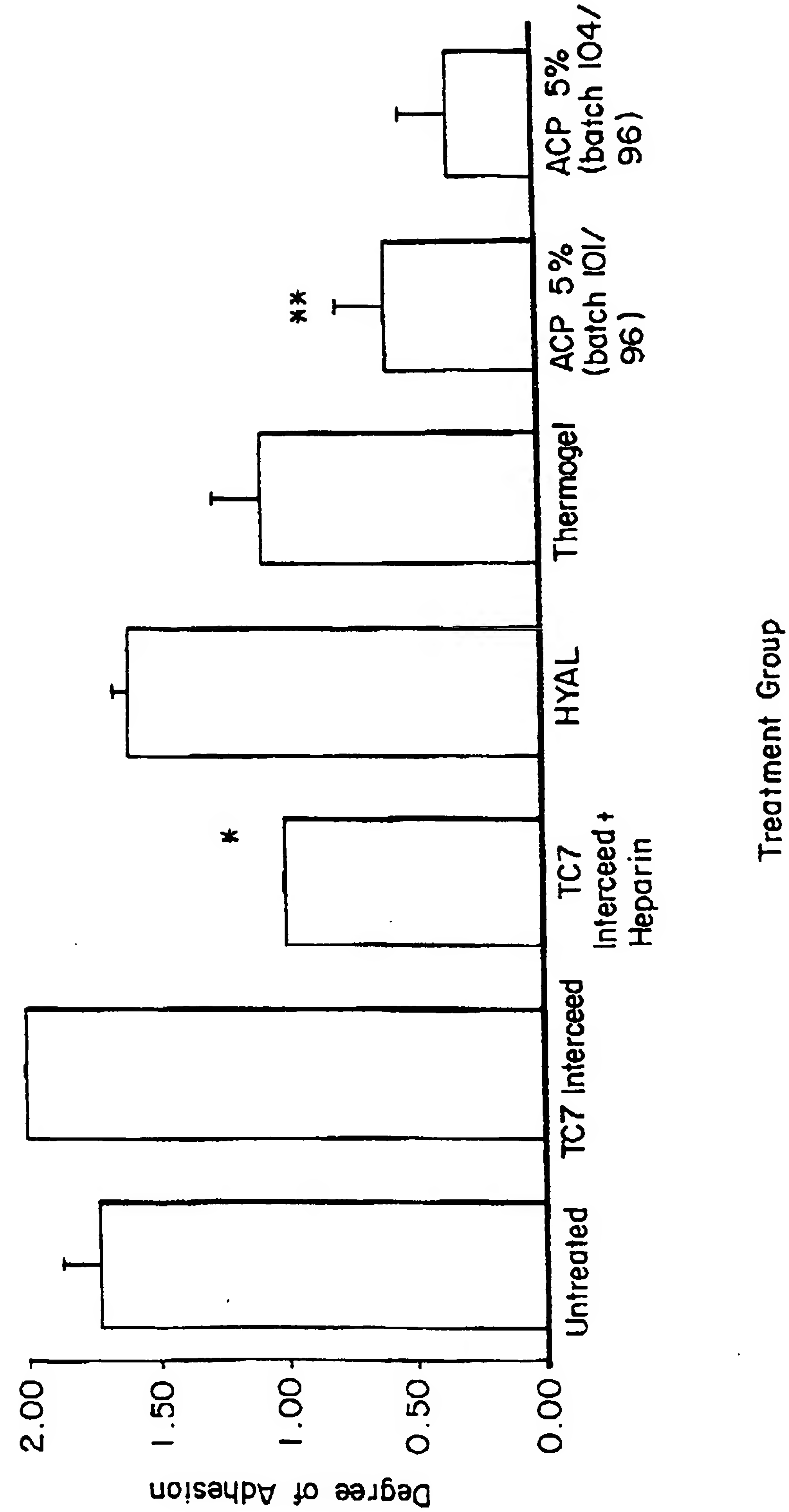
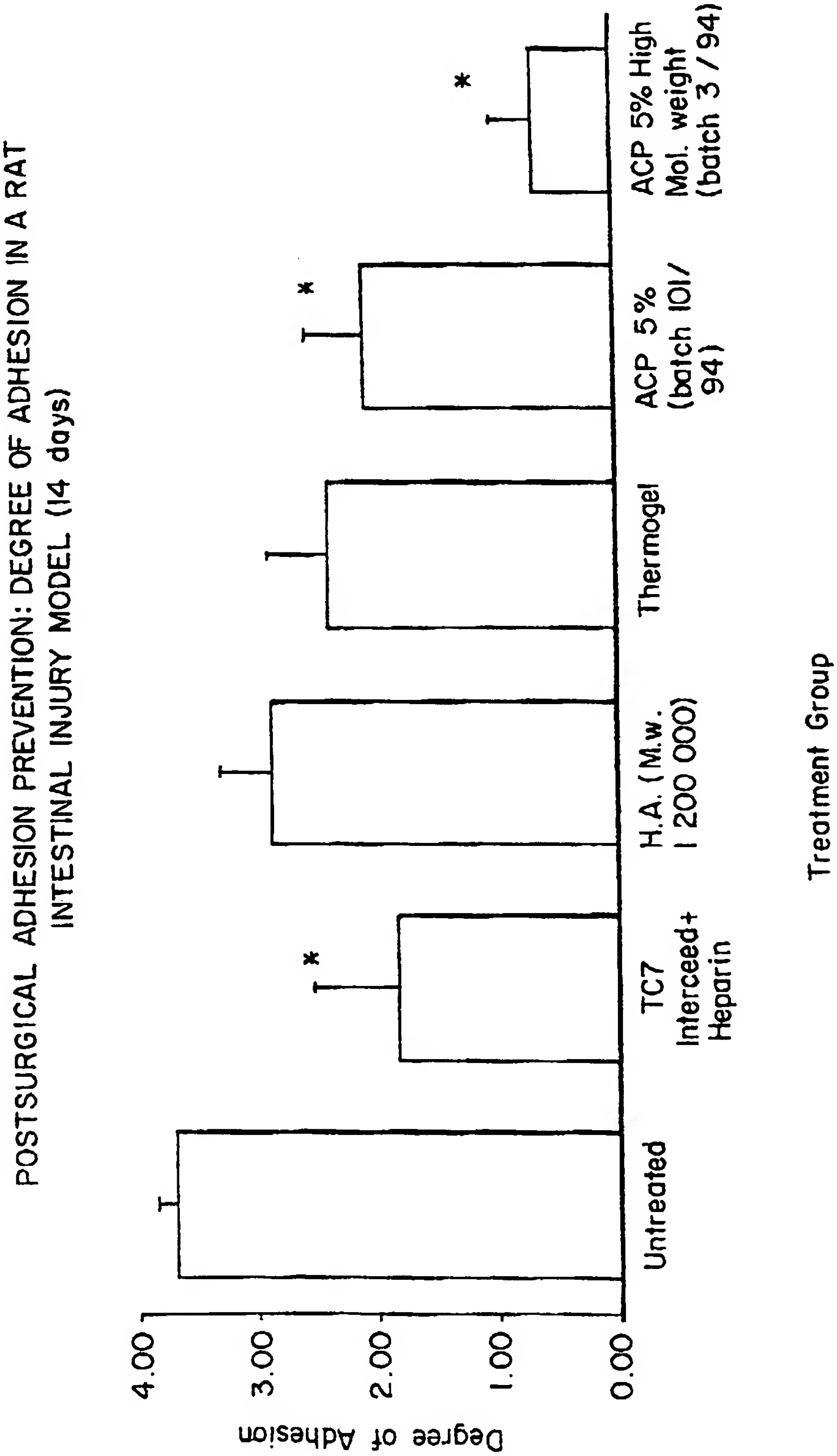


FIG. 10


POSTSURGICAL ADHESION PREVENTION: DEGREE OF ADHESION IN A RAT INTESTINAL INJURY MODEL (14 days)
Degree of Adhesion
0.00
1.00
2.00
3.00
4.00
Untreated
TC7 Interceed+ Heparin
H.A. (M.w. 1 200 000)
Thermogel
ACP 5% (batch 101/ 94)
ACP 5% High Mol. weight (batch 3 / 94)
*
Treatment Group